THE UNIVERSITY OF ALBERTA

ALKALOIDAL CONSTITUENTS OF *ELAEAGNUS COMMUTATA*

PART I. THE ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF ALKALOIDS FROM *E. COMMUTATA*

PART II. MASS SPECTRAL STUDIES OF SELECTED *beta*-CARBOLINES

by

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Alkaloidal Constituents of Elaeagnus Commutata", submitted by Gerald William Alexander Slywka, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Part I

A phytochemical investigation of Elaeagnus commutata Bernh. (E. argentea Pursh.) had previously revealed the presence of only quebrachitol and the methyl ester of ℓ-inositol. The isolation of alkaloids had not been reported.

The present investigation of the root bark of this plant revealed that there was approximately 1.12% total basic fraction which resulted in the isolation of two new alkaloids. One of these alkaloids has been identified as 1-isobutyl-1,2,3,4-tetrahydro-β-carboline. The structure has been proven by spectrophotometric methods and by synthesis. The structure of the second alkaloid is not completely known, although it is tentatively identified as 4'-isobutyl-3,3'-spiro-pyrrolidino-oxindole with a hydroxyl group on C-5.

Part II

The mass spectra of eight 1,2,3,4-tetrahydro-β-carbolines, two 1,2,3,4-tetrahydro-1-oxo-β-carbolines, four β-carbolines and a 3,4-dihydro-β-carboline have been interpreted and the proposed fragmentations have been proven by the use of accurate mass determination, deuterium labeling and the presence of metastable ions.

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PART I

THE ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF ALKALOIDS FROM *E. COMMUTATA*

INTRODUCTION AND STATEMENT OF PROBLEM

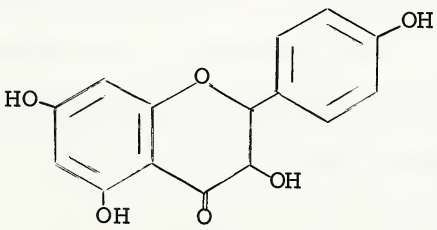
The isolation of alkaloids from the Elaeagnaceae family was not attempted until the late 1940's. Massagetov (1) reported the isolation of eleagnine (tetrahydroharmane) from Elaeagnus angustifolia, E. hortensis, E. orientalis and E. spinosa. In 1956, Platonova and his co-workers (2) continued the investigation of E. angustifolia and found two new β -carboline alkaloids, tetrahydroharmol and N-methyltetrahydroharmol. Arthur and collaborators (3) isolated two alkaloids from E. loureiri, however no further work was carried out on their identification. The Polish workers Lutomski, Nowicka and Adamska (4) showed that Hippophae rhamnoides contained harmalol, a dihydro-β -carboline. In 1968, Browne (5) investigated two species of Shepherdia (S. argentea and S. canadensis) and found two β -carboline alkaloids, tetrahydroharmol and shepherdine (6-hydroxytetrahydroharmane).

One of the members of the Elaeagnaceae family, Elaeagnus commutata (silver-berry or wolf willow) had not been investigated for the presence of alkaloids. Since this plant grows in abundance in Western Canada, it was planned to isolate, characterize and identify any alkaloids that might be present.

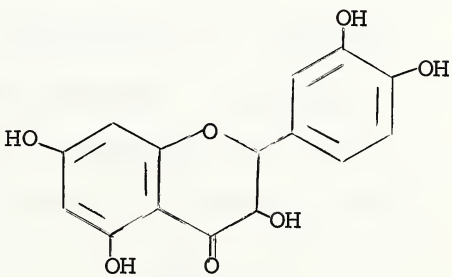
LITERATURE SURVEY

The plant family Elaeagnaceae consists of three genera which contain forty-five species of trees or shrubs more or less covered with minute silvery or brown scales. The genera, all worthy of representation in the garden, are Shepherdia, Hippophae and Elaeagnus (6).

Polyphenols, cyclitols and alkaloids are characteristic of the Elaeagnaceae family (7). Elaeagnus angustifolia, E. macrophylla, E. umbellata, Hippophae rhamnoides and H. salicifolia were investigated for their polyphenols (7). Kaempferol (I), quercetin (II), cumaric acid (III), and ellagic acid (IV) were detectable in all of the five species. E. angustifolia and E. macrophylla also contained sinapic acid (V) and ferulic acid (VI). E. umbellata contained sinapic acid.



I



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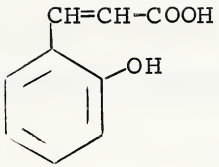

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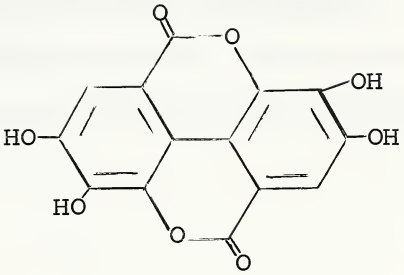

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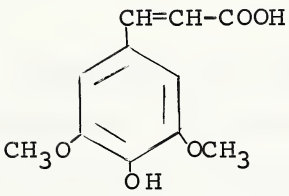

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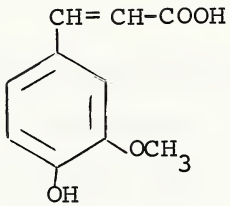
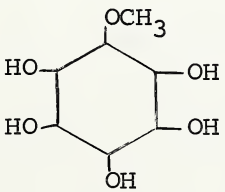


VI

Plouvier (8) investigated various species of the Elaeagnaceae family. Elaeagnus angustifolia, E. argentea, E. umbellata, E. multiflora, E. macrophylla, E. pungens, H. rhamnoides, H. salicifolia and S. argentea all contain quebrachitol (VII) or the methyl ester of ℓ-inositol (VIII).



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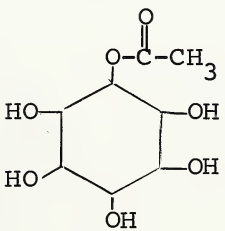
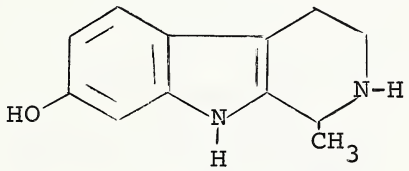


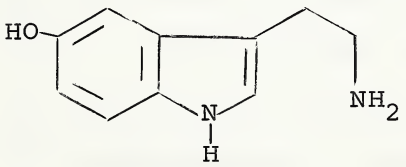
VIII

Shepherdia argentea (buffalo berry) is a perennial shrub or sometimes almost tree-form reaching eighteen feet, having red or yellow, edible berries. Browne (5) found the alkaloid tetrahydroharmol (IX) in this species.

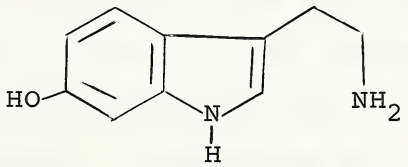


IX

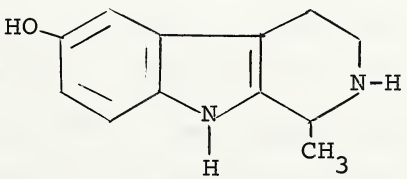
S. canadensis was also investigated by Browne (5). Tetrahydroharmol, serotonin (5-hydroxytryptamine) (X), 6-hydroxytryptamine (isolated as an artifact) (XI), and a new alkaloid, shepherdine (XII) were found in this species.



X

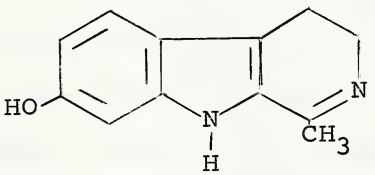


XI



XII

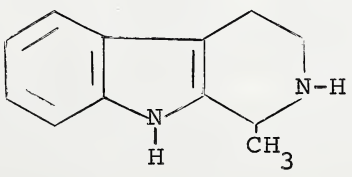
Petrova, Krants and Men'shikov (9) reported the isolation of serotonin (X) from the bark of Hippophae rhamnoides (sea buckthorn). Lutomski, Nowicka and Adamska (4) in a thin layer chromatographic investigation of the twigs and leaves of H. rhamnoides found harmalol (XIII).



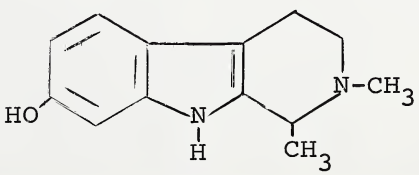
XIII

Elaeagnus angustifolia (Russian olive) is a non-leguminous nitrogen-fixing plant, not indigenous to Alberta but is found in shelter belts in the southern part of the province (10). It is a narrow-leaved oleaster widely distributed in the wild state in the U.S.S.R.. In Central Asia this plant is found in the form of trees and long bushes in dense thickets near rivers. In 1946, Massagetov (1) reported the isolation of two alkaloids from the bark of E. angustifolia, E. hortensis, E. orientalis and E. spinosa. One was a crystalline alkaloid, which was soluble in ether, chloroform and ethanol; slowly soluble in water, and was optically inactive. It had the composition $C_{12}H_{14}N_2$ and melted at 180-181.5°. This alkaloid was called eleagnine (XIV). Men'shikov, Gurevich and Samsonova (11) showed that the alkaloid eleagnine was the racemic form of tetrahydroharmane. In 1955, Platonova, Kuzovkov

and Massagetov (2) continued the investigation of E. angustifolia. The bark on extraction with alcohol, yielded a total basic fraction (0.2% of the weight of the dry bark) in which paper chromatography revealed the presence of three alkaloids. Alkaloid A had a composition corresponding to the formula $C_{12}H_{14}ON_2$, and its ultraviolet spectrum resembled that of tetrahydroharmine. The infrared spectrum showed the presence of the OH and NH groups. The base possessed phenolic properties. This data showed that the structure molecule of alkaloid A was based on the tetrahydroharmane nucleus and contained a phenolic hydroxyl group. Alkaloid A was shown to be tetrahydroharmol (IX) by comparing it with the product obtained when harmol was reduced with sodium in anhydrous alcohol. Alkaloid B had the composition $C_{13}H_{16}ON_2$ and differed from that of alkaloid A in that it contained a NCH_3 group. It also showed phenolic properties. On methylation of tetrahydroharmol with methyl iodide, a mixture of products was formed, in which the presence of alkaloid B was established by paper chromatography. Alkaloid B proved to be N-methyltetrahydroharmol (XV). Alkaloid C was identified as eleagnine, which had been reported earlier.



XIV



XV

Figure I

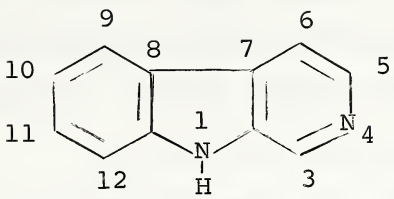
Elaeagnus commutata Bernh. (E. argentea Pursh.)

Arthur, Chan, Loo, Tam and Tung (3) extracted the dried leaves of E. loureiri with cold ethanol. Two bases were found that were different from those recorded in other Elaeagnus species. Elaeagnus alkaloids E_1 and E_2 both were recrystallized from light petroleum ether and had melting points respectively of 117-120° and 124-126.5°. No further work has been reported on the identification of these two alkaloids.

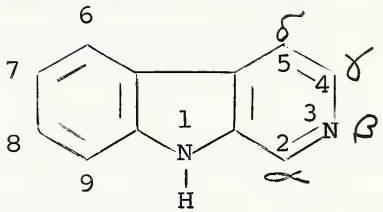
Elaeagnus commutata (silver-berry or wolf willow) is a shrub from two to twelve feet tall widely distributed throughout Canada. It is common in Western Canada growing on dry hillsides, sandy banks and open fields. Although this shrub derives its common name from the grayish-white color of its berries, every part of it, stems, branches, leaves and flowers, are covered with the same lustrous coating of silvery scales. The small tubular flowers are lemon yellow within and have an overpowering heavy scent. The berries are dry and mealy and contain a single stony seed marked with light yellow striped grooves (12). Corns and Schraa (13) studied the mechanical and chemical control of silver-berry on the native grassland. Moore (10) and Bond (14) both investigated E. commutata from the aspect of its ability to fix nitrogen even though it is not a legume. Very little phytochemical work has been carried out on this species.

Most of the bases isolated from the Elaeagnaceae family are tetrahydro- β-carbolines and β-carbolines

(harmala alkaloids) (1,2,3,4,5).



1



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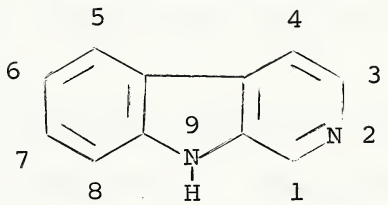


3

The nomenclature used to describe the fused benzene-pyrrole-pyridine system has been repeatedly modified since Perkin and Robinson introduced the name carboline for the ring system, which was encountered for the first time in the harmala alkaloids. In the early years, the parent compound of the series, whose trivial name was norharmane, was referred to as 4-carboline and numbered as in 1. Later, the numbering of the carboline system was modified to that shown in 2, and the position of the basic nitrogen in the pyridine ring was designated by a Greek letter. Harmine thus became 8-methoxy-2-methyl- β-carboline.

According to "The Ring Index" the system is classified

as pyridoindole and numbered as in 3 (harmine would be 7-methoxy-1-methyl-9H-pyrido (3,4-b) indole). This is the nomenclature adopted by Chemical Abstracts Index, according to which β-carboline is designated 9H-pyrido (3,4-b) indole (15).

The numbering system used in 3 was introduced also in conjunction with the carboline nomenclature. This is the system which, at the present time, appears to be most widely adopted. The Editor of the "Journal of the Chemical Society" in 1952 and the Definitive I.U.P.A.C. (1957) adopted the numbering for the β-carboline shown in structure 3 (16).

The harmala alkaloids crystallize easily and have moderately high melting points. The pyridine nitrogen is basic, while the indole nitrogen is virtually non-basic. Most of the β-carbolines and their homologues give a brilliant bluish-violet fluorescence in dilute solution and have a characteristic absorption in the ultraviolet (17, 18).

Tetrahydroharmane (1-methyl-1,2,3,4-tetrahydro-β-carboline) and the related alkaloids may be synthesized by the condensation of tryptamine or a substituted tryptamine with acetaldehyde. Other β-carbolines can be derived by substituting a methyl group on the basic nitrogen followed by dehydrogenation of the tetrahydropyridine ring (19) (see Figure II).

The biosynthesis of naturally occurring carbolines

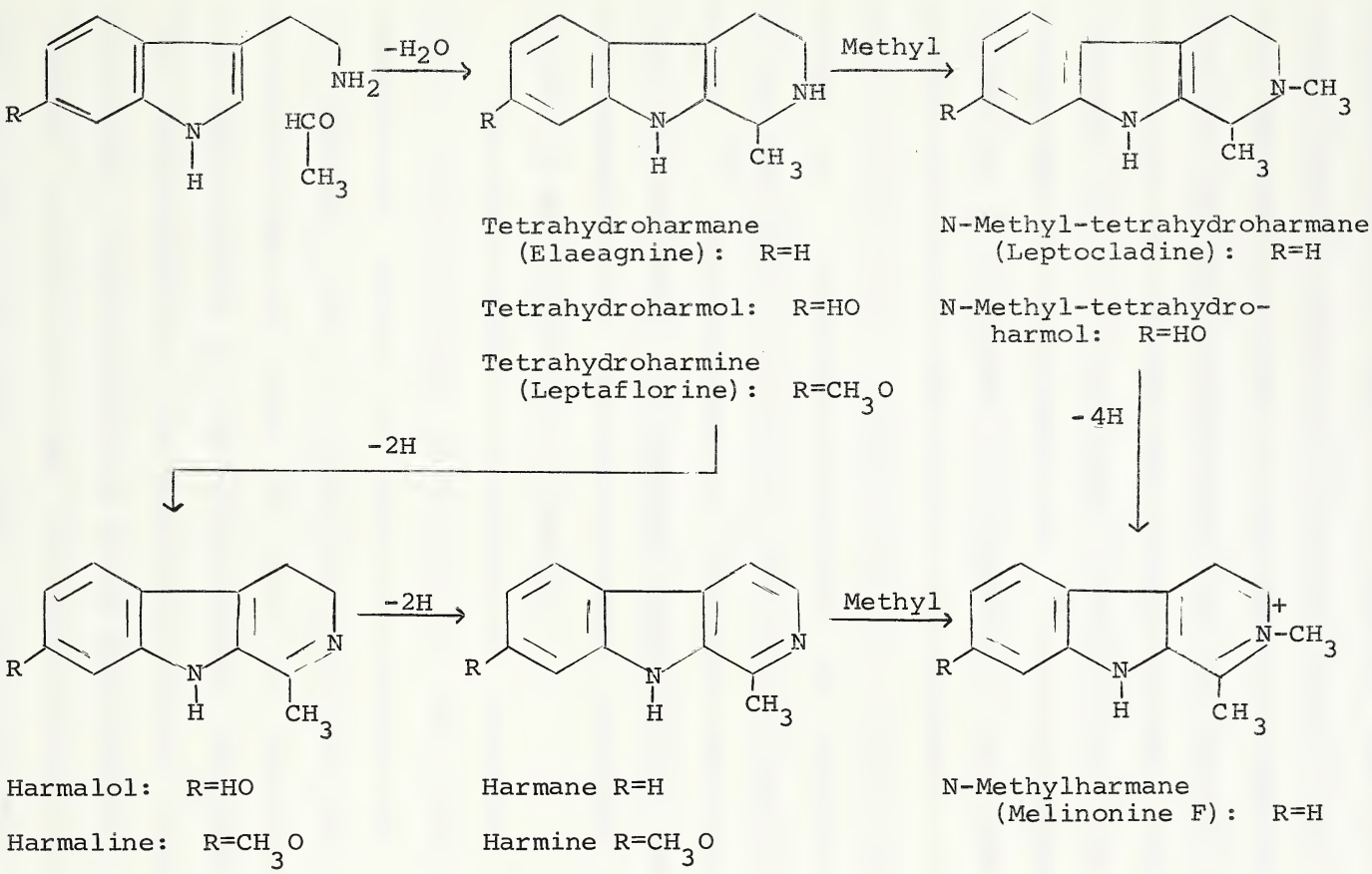


Figure II. Harmala Alkaloids

were studied as early as 1919, when Perkin and Robinson noted that harmalol, harmine and harmaline, the only naturally occurring carboline derivatives known at that time, were structurally related to tryptophan and suggested that the bases were biogenetically derived from the amino acid by way of a hypothetical hydroxytryptophan. The formation of 1,2,3,4-tetrahydro- β -carbolines as the initial step in the biogenesis of β-carboline alkaloids, by reaction of tryptophan or tryptamine or the corresponding hydroxylated derivatives with aldehyde or α -keto acids, derived from α -amino acids, is a widely held concept (16). O'Donovan and Kenneally (20) proved this earlier hypothesis by isolating radioactive eleagnine when dl-(α -^{14}C) tryptophan and (1-^{14}C) acetate were fed, in separate experiments, to Elaeagnus angustifolia. Unambiguous degradations of the alkaloid showed tryptophan and acetate to be precursors of eleagnine.

The psychotropic or hallucinogenic properties of plants containing harmala alkaloids have been extensively investigated. Naranjo (21) stated that harmine was the only compound in the β -carboline group which had hallucinogenic properties. Gunn (22) found that harmaline was also hallucinogenic at high dosage levels. He showed that tetrahydroharmine, the reduction product of harmine, was similar in such properties to its more saturated homologs, but was three times less active than harmaline. Mental patients when given harmine showed impairment of

contact, attention, grasp, responsiveness and concentration. Subjects also noted some vertigo, lightheadedness, ataxia, perceptual disturbances and hallucinations (23).

The formation of a 1,2,3,4-tetrahydro- β -carboline in the pineal gland tissue of rats has been postulated by McIsaac (24). It was reported that adrenoglomerulotropin, a factor specific for aldosterone secretion in the pineal gland, might be 6-methoxy-1-methyl-1,2,3,4-tetrahydro- β -carboline. He showed that the presence of iproniazid inhibited the degradation of 5-methoxytryptamine by monoamine oxidase (MAO) to 5-methoxyindole acetic acid. Similarly, the degradation of acetaldehyde was prevented by Antabuse and the condensation of 5-methoxytryptamine and acetaldehyde to form 6-methoxy-1-methyl-1,2,3,4-tetrahydro- β -carboline was thus favoured. Supniewski and Miszkal (25) confirmed the hypothesis by synthesizing 6-methoxy-1-methyl-1,2,3,4-tetrahydro- β -carboline and comparing it with adrenoglomerulotropin. McIsaac, Khairallah and Page (26) suggested that in certain psychotic conditions N_b-acetyl-5-methoxytryptamine might give rise to 6-methoxy-1-methyl-3,4- β -carboline by a metabolic analog of the Bischler-Napieralski reaction.

The alkaloid harmine and related natural tricyclic compounds belong to a group of extremely potent competitive and reversibly acting inhibitors of enzymatic deamination of biogenic monoamines (27). Ho, McIsaac, Walker and Estevez (28) synthesized a number of tetrahydro- and

aromatic β-carbolines, mostly with a methyl substituent at various positions. Their *in vitro* inhibitory activities on monoamine oxidase were evaluated. It was shown that substitution of a methyl group at the N-9 nitrogen (indole N) of tetrahydro-β-carboline gave a potent competitive inhibitor of the enzyme. Methyl groups at C-1 of both tetrahydro- and aromatic β-carbolines generally reduced the potency, whereas introduction of a methyl group at the N-2 nitrogen of tetrahydro-β-carboline gave a compound of equal activity to that of the substitution of a methyl group at the N-9 nitrogen. Ho, McIsaac and Walker (29) also synthesized tetrahydro-β-carbolines with alkyl or aralkyl substitution on the N-9, alkyl on N-2, and alkyl on both N-2 and N-9 positions. The N-9-methyltetrahydro-β-carboline was found to be a competitive inhibitor of the enzyme, being three times more active than iproniazid. The N-2 nitrogen of the tetrahydro-β-carboline could be substituted by alkyl groups as large as propyl without affecting the inhibitory activity.

Both hallucinogenic activity and inhibition of MAO form the basis of the therapeutic use of the harmala alkaloids. However, various β-carbolines including 1-isobutyl-1,2,3,4-tetrahydro-β-carboline were tested as potential analgesics (30). Some dihydro- and aromatic β-carbolines have been found to possess depressant activity, as well as antiparasitic, anti-inflammatory,

and anorexigenic properties (31, 32). Harmine and harmaline have also been used as anthelmintics, presumably through an action on the muscles of the tapeworm (18).

DISCUSSION

The large-scale extraction procedure was carried out in a soxhlet extractor. The finely powdered root bark was pre-extracted with petroleum ether (bp 30-60°) to remove lipids and some of the coloring matter. The plant material was then extracted with 95% ethanol until evaporation of a portion of the extract left little or no residue. The ethanol extract was concentrated to a small volume and was dripped into 2% aqueous hydrochloric acid under vacuum to obtain the alkaloids in the aqueous acid solution. A precipitate of acid insoluble materials was filtered and a dark brown filtrate was extracted with chloroform at pH 1, 8 and 11. The chloroform extracts gave, after evaporation in vacuo, an acidic fraction E.1, two basic fractions E.2 and E.3 which thin layer chromatography (TLC) showed to be virtually the same (Table 6) and a quaternary fraction E.4.

Column elution chromatography of extract E.1 on neutral alumina, activity III, using various solvent combinations of increasing polarity gave poor separation of the components. Since TLC proved to be extremely efficient in the detection of the compounds and effected good separation, it was decided to use TLC in a preparative manner using n-hexane:chloroform:methanol - 40:20:11 as the solvent. Compounds were detected on the TLC plates by means of their fluorescence under ultraviolet light. The zones were marked and removed after drying

by the "micro vacuum cleaner" technique. Elution of the silica gel either in the cold or by a microsoxhlet extraction gave chromatographically pure Compound 1 (R_f 0.37).

In order to obtain a crystalline compound, methanolic hydrogen chloride was added to a methanol solution of Compound 1, resulting in a crystalline hydrochloride salt (mp 257-259°). All spectrophotometric studies were performed on this hydrochloride salt, which was subsequently shown to be 1-isobutyl-1,2,3,4-tetrahydro-β-carboline HCl (Figure III).

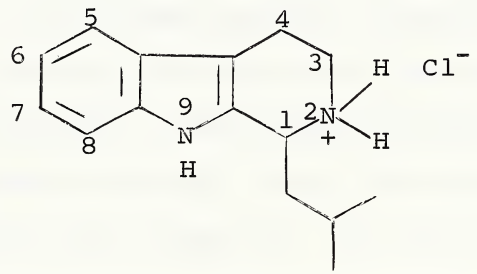


Figure III: 1-Isobutyl-1,2,3,4-tetrahydro-β-carboline HCl

The presence of an indole chromophore (33) is indicated by the ultraviolet (UV) spectrum $\lambda_{max}^{ethanol}$ 223 mμ (log ε = 4.67)+, 273 mμ (log ε = 3.85), 279 mμ

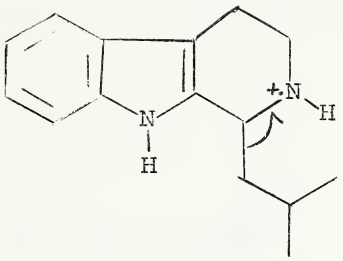
+ The molecular weight of Compound 1 was established as 228 from an accurate mass of the molecular ion. The calculation of log ε was based on the hydrochloride salt of Compound 1 (molecular weight, 264).

(log ε = 3.86), 289 mμ (log ε = 3.80). There was no bathochromic shift upon the addition of either acid or base. Further information was obtained from the infrared (IR) spectrum (Figure VIII) of Compound 1 HCl which showed a characteristic indolic N-H band at 3200 cm^{-1}.

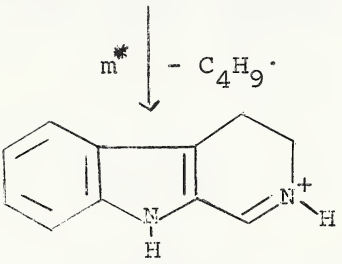
The nuclear magnetic resonance (NMR) spectrum (Figure IX) showed a singlet at τ -1.1, a broad band at τ 0.30 and τ 5.38, a four proton multiplet of aromatic protons at τ 2.75, three multiplets at τ 6.75, τ 7.00 and τ 7.95, and a six proton triplet at τ 8.98. The singlet at τ -1.1, which was slowly exchanged with D_2O, represented the proton attached to the indole nitrogen. A broad band at τ 0.30 integrating for 2 protons, which exchanged readily with D_2O, was due to the protons attached to the N-2 nitrogen. One of these protons was attached to a secondary nitrogen, while the other proton appeared to be due to salt formation. In the free base of the methyl ester of tetrahydronorharmane carboxylic acid, this did not occur, however the presence of two singlets, one at τ 1.85 and τ 7.80, which exchanged readily with D_2O, were observed (Table 8). It would appear that the protons attached to the N-2 nitrogen in the salts were shifted downfield. Two multiplets at τ 6.75 and τ 7.00 were indicative of the $-CH_2-CH_2-$ group in the piperidine portion of the molecule. The methine and methylene protons of the isobutyl side chain gave a multiplet centered at τ 7.95 and the C-1 proton showed a

broad band at τ 5.38. The proton at C-1 was not present in the methyl ester of tetrahydronorharmane carboxylic acid, but it did appear in all three 1,2,3,4-tetrahydro-β-carbolines (Table 8). The six proton triplet at τ 8.98 was due to the presence of two methyl groups on the isobutyl chain. The isopropyl side chain 1-isopropyl-1,2,3,4-tetrahydro-β-carboline gave a doublet of doublets centered at τ 8.82 and τ 9.09 corresponding to two methyl groups (Table 8). When the length of the side chain is increased by a methylene group to give the isobutyl side chain, the doublets overlap to give a triplet. In both compounds the methyl groups of the isopropyl moiety are magnetically non-equivalent and give rise to distinct signals. Sorenson (34) and Bowman (35) have observed the nuclear magnetic non-equivalence of methyl groups in the isopropyl moieties of various alcohols and ketones.

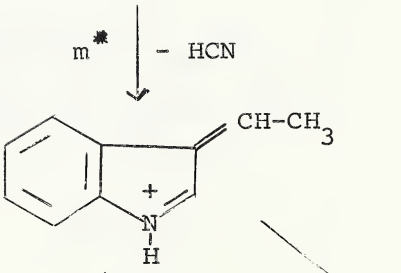
Further information was obtained by examining the mass spectral fragmentation pattern (Figure X) of Compound 1. The main fragmentation pathway (scheme 1) occurred through the cleavage of the isobutyl side chain as a radical resulting in the formation of the base peak at m/e 171. This fragmentation was followed by the loss of a HCN molecule, a H radical and an acetylene molecule to give peaks at m/e 144, 143 and 117 respectively. The peak at m/e 117 may also arise by the loss of a HCN molecule from m/e 144, since m/e 117 was approximately



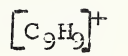
m/e 228



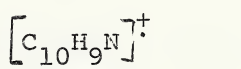
m/e 171



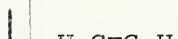
m/e 144

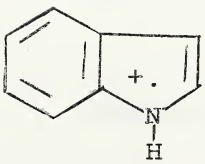


m/e 117



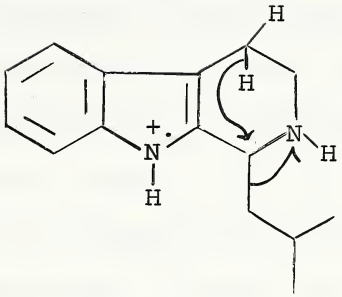
m/e 143





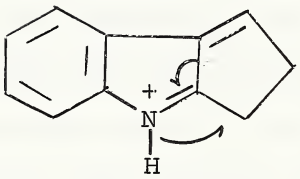
m/e 117

SCHEME 1



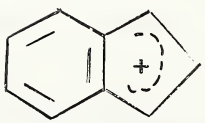
m/e 228

m* $-C_4H_9NH\cdot$



m/e 156

m* $-CH_3CN$



m/e 115

SCHEME 2

66 per cent C_8H_7N and 33 per cent C_9H_9 (accurate mass determinations).

A secondary pathway (scheme 2) for the fragmentation of Compound 1 involved a rearrangement and then an expulsion of an RNH radical from the molecular ion to give an ion of mass m/e 156. A subsequent loss of a $CH_3C{\equiv}N$ molecule gave a peak at m/e 115.

Although C-1 is an asymmetrical carbon atom in 1-isobutyl-1,2,3,4-tetrahydro- β -carboline giving the possibility of 2 isomers, the compound was found to be optically inactive. The isolated compound would therefore be a racemic mixture.

Further proof that Compound 1 was 1-isobutyl-1,2,3,4-tetrahydro- β -carboline was provided when no melting point depression was shown upon the addition of the synthesized compound to the isolated alkaloid. The R_f values (Table 4) obtained from TLC (n-hexane:chloroform: methanol - 40:20:11, n-butanol:acetic acid:water - 4:1:1, chloroform:methanol - 9:1, n-propanol:ammonium hydroxide - 8:2), retention times for gas chromatography, NMR spectra (Figure IX) and IR spectra (Figure VIII) were identical for the two compounds.

Compound 2, the major alkaloid, a creamy-white powder, was precipitated from extract E.2 upon the addition of acetone. It was crystallized from ethanol and had a melting point of 250-252°. The crystalline base was insoluble in water, chloroform, ether, carbon tetra-

chloride, acetone and 10% sodium bicarbonate. It was soluble in dilute sulphuric acid and 5% sodium hydroxide. It dissolved in methanol or ethanol upon the application of heat. The molecular formula, $C_{15}H_{20}N_2O_2$ (260), was established by elemental analysis. An analysis of a sample revealed that there were no $O\text{-}CH_3$ or $N\text{-}CH_3$ groups present. A Kuhn-Roth C-methyl determination showed that one $C\text{-}CH_3$ group was present, although a geminal dimethyl group would also give only one $C\text{-}CH_3$ group, since only one mole of acetic acid can be formed. Valser's reagent and the ferric chloride test gave positive results, while the 2,4-dinitrophenylhydrazine test was shown to be negative. It would appear that Compound 2 might be a phenolic alkaloid and not a simple ketone or aldehyde. Subsequent spectrophotometric studies indicate that compound two might have the following structure (Figure IV).

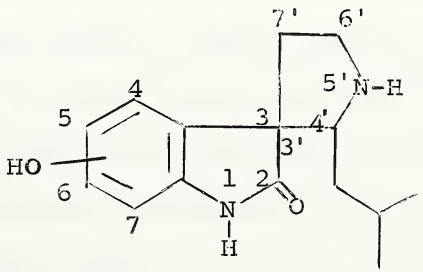
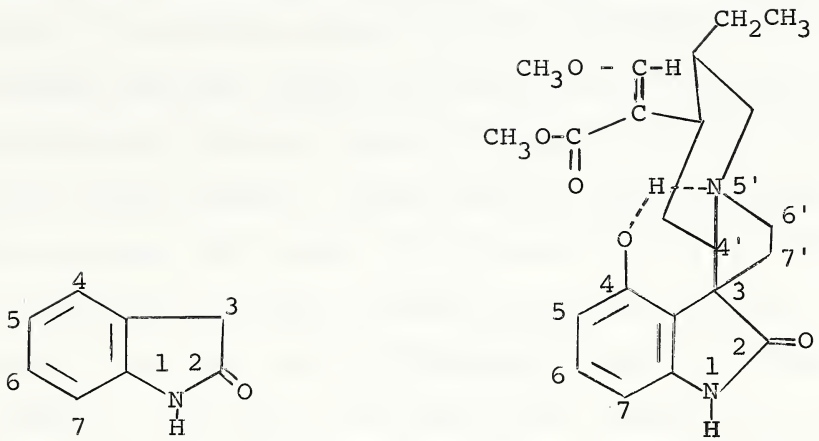


Figure IV: Compound 2

The UV spectrum of Compound 2 in ethanol showed high intensity maxima at 223 mμ (log ε = 4.42)+, 260-262 mμ (log ε = 3.58), 284 mμ (log ε = 3.52) and an inflection at 294 mμ (log ε = 3.41). This resembles the spectra of naturally occurring compounds containing the N-acyldihydroindole moiety (36), however it was shown by micro-analysis, NMR and formation of a triacetyl derivative that neither of the two nitrogens were involved in tertiary amide linkages and both contained replaceable hydrogens. The spectrum also resembled that of a 6-hydroxyoxindole (257 mμ (log ε = 3.68), 288 mμ (log ε = 3.44) and inflection at 294.5 mμ (log ε = 3.36) in neutral solution (37), although Compound 2 contained a band at 223 mμ and a maximum at 260-262 mμ instead of 257 mμ. A bathochromic shift (210 mμ (log ε = 4.40), an inflection at 220 mμ (log ε = 4.31) and a band at 300 mμ (log ε = 3.63)) was shown when sodium hydroxide was added to a solution of Compound 2. This type of shift is observed upon conversion of a phenol to its anion and thus was confirmatory evidence for a phenolic hydroxyl group. The UV spectra of most oxindoles contain a band in the region 242-260 mμ although crassanine (38) and kisantine (39) which are both 5,6-dimethoxy substituted oxindoles (Figure V) have a band between 268-275 mμ.

+The calculation of log ε was based on the molecular weight of Compound 2, which was shown to be 260 by analytical analysis.



Oxindole

Rotundifoline

Figure V: Structure of Oxindole and Rotundifoline

The infrared spectrum of Compound 2 (Figure XI) in nujol showed a N-H band (3225 cm^{-1}), a weak broad band (2450 cm^{-1}), a carbonyl band (1710 cm^{-1}), and a band (1630 cm^{-1}) characteristic of aromatic absorption. The band at 3225 cm^{-1} is characteristic of an indolic N-H, although it may be due to an overlapping of an OH and NH absorption. Rotundifoline (40) has a peak centered at 2500 cm^{-1} which is due to OH·····N bonding. Models indicate that the C-4 (Figure V) is the only position where the hydroxyl group may be intramolecularly bonded with the N-5' nitrogen. This bonding is shown by insolubility in 5% sodium hydroxide for rotundifoline and not for its isomer isorotundifoline. Compound 2 has the weak broad band at 2450 cm^{-1} which appears to be due to a intermolecular bonding between the carbonyl oxygen of one

molecule and the hydrogen on the indole nitrogen of another molecule. A dilution study in carbon tetrachloride or chloroform solution may have indicated the presence of intermolecular bonding, however Compound 2 was not soluble in either solvent. There might be intramolecular bonding between the oxygen of the carbonyl and the hydrogen on the N-5' nitrogen, however it appears from observing a molecular model of Compound 2 that this does not happen. The lack of an appreciable change in the UV spectrum of the hydrogen bonded isomer (rotundifoline) in alkaline solution compared with neutral solutions contrast with the bathochromic shift of the 290 mμ peak (18 mμ) in the non-intramolecularly bonded isorotundifoline. There is a very appreciable change in the UV spectrum of Compound 2 in alkaline solution compared with neutral solution and this contrasts the lack of a bathochromic shift with rotundifoline. Upon acetylation the N-acetyl derivative for rotundifoline was formed, since the OH····N bond was still present in this compound. Acetylation of Compound 2 indicated that there are 3 replaceable hydrogens and one of these would have to be the phenolic hydroxyl group. It can be concluded that the phenolic hydroxyl group does appear not to be intramolecularly bonded and probably is not on C-4. The carbonyl band at 1710 cm^{-1} is in agreement with an oxindole structure since it was too high for a six-membered lactam (strychnine, 1665 cm^{-1}), or an acyl amide (diaboline, 1660 cm^{-1}) (41).

Compound 2 was not soluble in chloroform and a NMR spectrum (Figure XII) was determined in deuterated dimethyl sulfoxide. A proton at τ -0.02, which exchanged with D_2O, indicated the proton from the oxindole nitrogen. A one proton multiplet at τ 3.01 and a two proton multiplet at τ 3.70 were due to the three aromatic protons. The six proton triplet at τ 9.28 was similar to that of 1-isobutyl-1,2,3,4-tetrahydro-β-carboline (τ 8.98) which indicates the presence of the two methyl groups on the isobutyl side chain.

The NMR spectrum of Compound 2 indicates that the hydroxyl group is located on C-5. The one proton multiplet at τ 3.01 is most reasonably assigned to the proton at C-7, since this proton would be deshielded by the nitrogen atom of the oxindole ring and would be expected to be the furthest downfield. The two proton multiplet at τ 3.70 is observed upfield due to the shielding effect of the hydroxyl group.

If the hydroxyl group is at C-5 then the proton at C-7 will show ortho coupling with the proton at C-6 and para coupling with the proton at C-4. The proton at C-4 would show meta coupling with the proton on C-6 and para coupling with the proton at C-7. The proton on C-6 would show ortho coupling with the proton at C-7 and meta coupling with the proton at C-4. This is observed in the spectrum of Compound 2. The two protons at C-4 and C-6 were shielded by the hydroxyl group and the proton at C-7 would be de-

shielded by the nitrogen atom of the oxindole ring.

If the hydroxyl group is at C-6 then the proton at C-7 would have meta coupling with the proton at C-5 and para coupling with the proton at C-4. This does not exist in the spectrum of Compound 2 at τ 3.01, but ortho coupling (J = 8 cps.) was shown to exist.

If the hydroxyl group was attached to C-4 then the C-7 proton would show ortho coupling with the C-6 proton and meta coupling with the C-5 proton. This possibility does exist in Compound 2 although it is difficult to establish if the coupling is ortho-para or ortho-meta (Figure XII). The C-6 proton would have ortho-ortho coupling with the C-7 and C-5 protons. No ortho-ortho coupling occurred in the spectrum.

Since C-7 is the only position on the aromatic ring where a proton can be deshielded, the possibility of the hydroxyl group occupying C-7 would not be probable.

It can be concluded from the NMR evidence that the hydroxyl group of Compound 2 would be located on C-5.

The NMR spectrum of 5-hydroxyoxindole and 5-hydroxyindole were run in deuterated DMSO. The aromatic region of 5-hydroxyindole was more complex than that in the 5-hydroxyoxindole (Table 8). This was due to the presence of two more aromatic protons. The aromatic region (Figure VI) of the 5-hydroxyoxindole was similar to that of Compound 2, although the three aromatic protons are close together and not separated. It would appear from above data that Compound 2 was a phenolic oxindole with the phenolic hydroxyl substituted at C-5.

The characteristic mass spectral fragmentation pattern of oxindole alkaloids offers a convenient means of their characterization. The mass spectra of tetra- and pentacyclic oxindoles occur through homolytic rupture of the 3'-4' and 6'-7' bonds, yielding a molecule and a radical. In most cases, the base peak is derived from the alicyclic portion of the molecule. However the molecular ion and base peak of crassanine (38) are the same. The spectrum of Compound 2 suggested a tricyclic oxindole since the number of double bond equivalents was seven. The suggested structure for Compound 2, yielding a molecule a and a radical (m/e 99). The latter fragment decomposed further by a

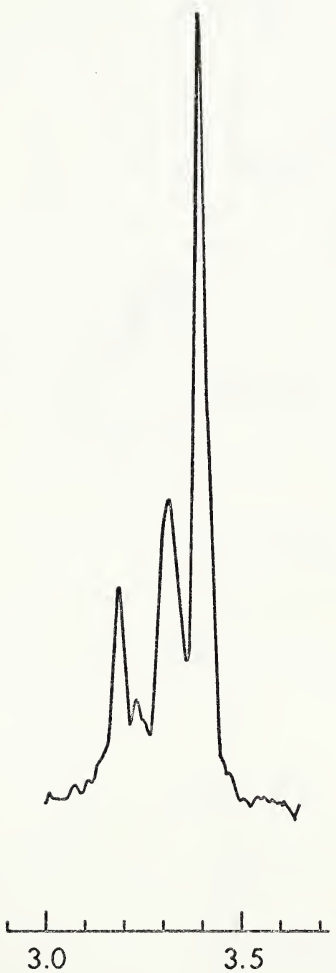
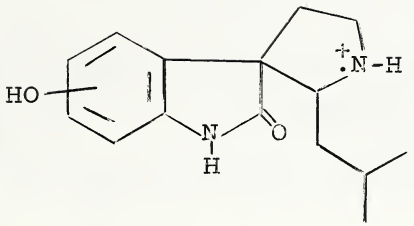
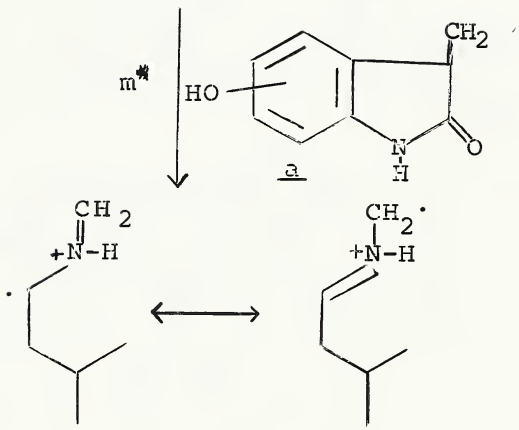


Figure VI

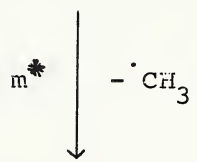
NMR (60 Mc) Spectrum of the Aromatic Protons of 5-Hydroxyoxindole



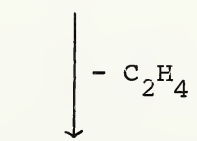
m/e 260



m/e 99 (b)

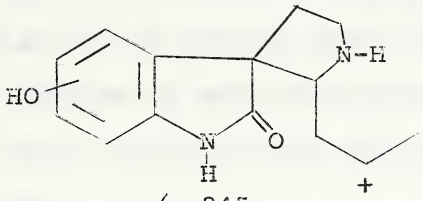


m/e 84

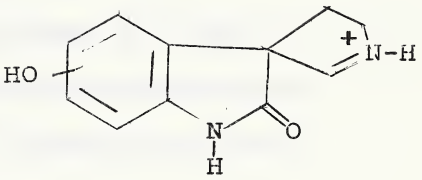


m/e 56

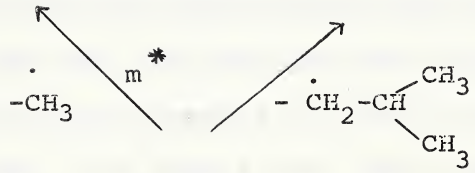
SCHEME 3

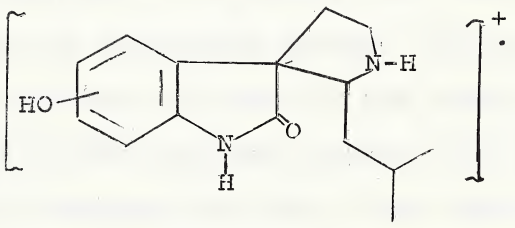


m/e 245

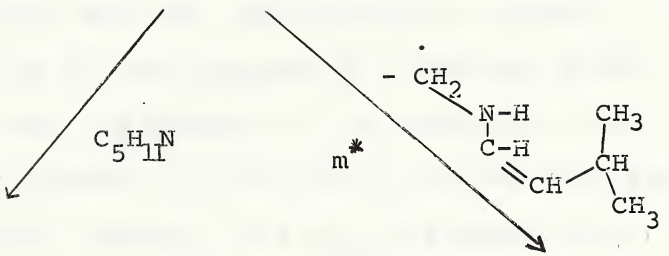


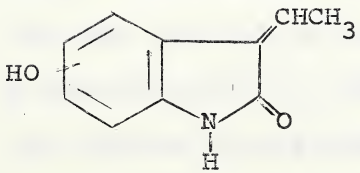
m/e 203



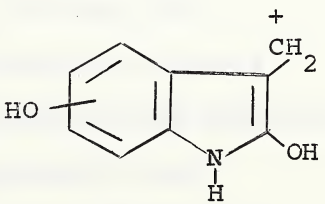


m/e 260





m/e 175



m/e 162

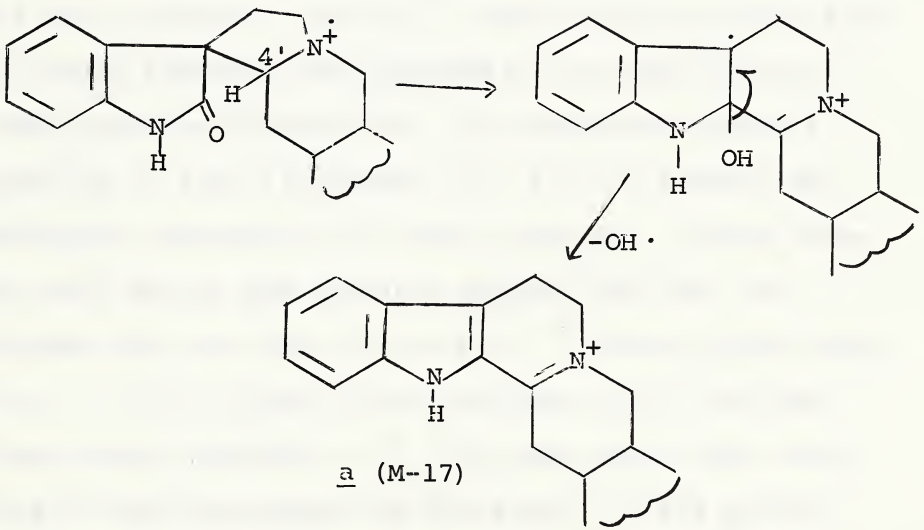
SCHEME 4

loss of a methyl radical to the base ion at m/e 84. The subsequent loss of a C_2H_4 molecule resulted in the formation of an intense peak at m/e 56 (scheme 3). The presence of metastable ions and accurate mass measurements confirmed this mass spectral fragmentation pathway.

Fragments containing the indole moiety are found at m/e 130, 144-146 and 159. The m/e 130 and 144 peaks are ubiquitous among the mass spectra of indole and dihydroindole alkaloids. The peak at m/e 159 is due to the retention of the tryptamine bridge. The assignments are unequivocal and are analogous to the reported shift of 30 mass units in the acricine oxindole and 60 mass units in that of carapanaubine due to the additional methoxy function in the aromatic ring (42). In Compound 2, there are peaks at m/e 146, 160-162 and 175, which may be accounted for by the presence of a hydroxyl group on the aromatic ring. In scheme 4 it is shown that the molecular ion can fragment by the loss of various radicals. The loss of a methyl, isobutyl or a $C_6H_{12}N$ radical from the molecular ion gave peaks at m/e 245, 203 and 162. The loss of a $C_5H_{11}N$ molecule from the molecular ion gave a peak at m/e 175. The direct fragmentation of the molecular ion to ions at m/e 245 and 162 were confirmed by the presence of metastable ions. The composition of the ion at m/e 175 was confirmed by an accurate mass measurement.

Another characteristic feature of mass spectra of

the oxindoles is a small but easily recognizable (M-17) peak due to the loss of a hydroxy radical. Since the fragment occurs in compounds which do not contain the hydroxyl function, its formation must involve the loss of the lactam oxygen together with a hydrogen atom. Deuterium labeling has shown that this hydrogen atom comes from C-4'. The following mechanism leading to species a is a plausible representation for the (M-17) fragment proposed (43).



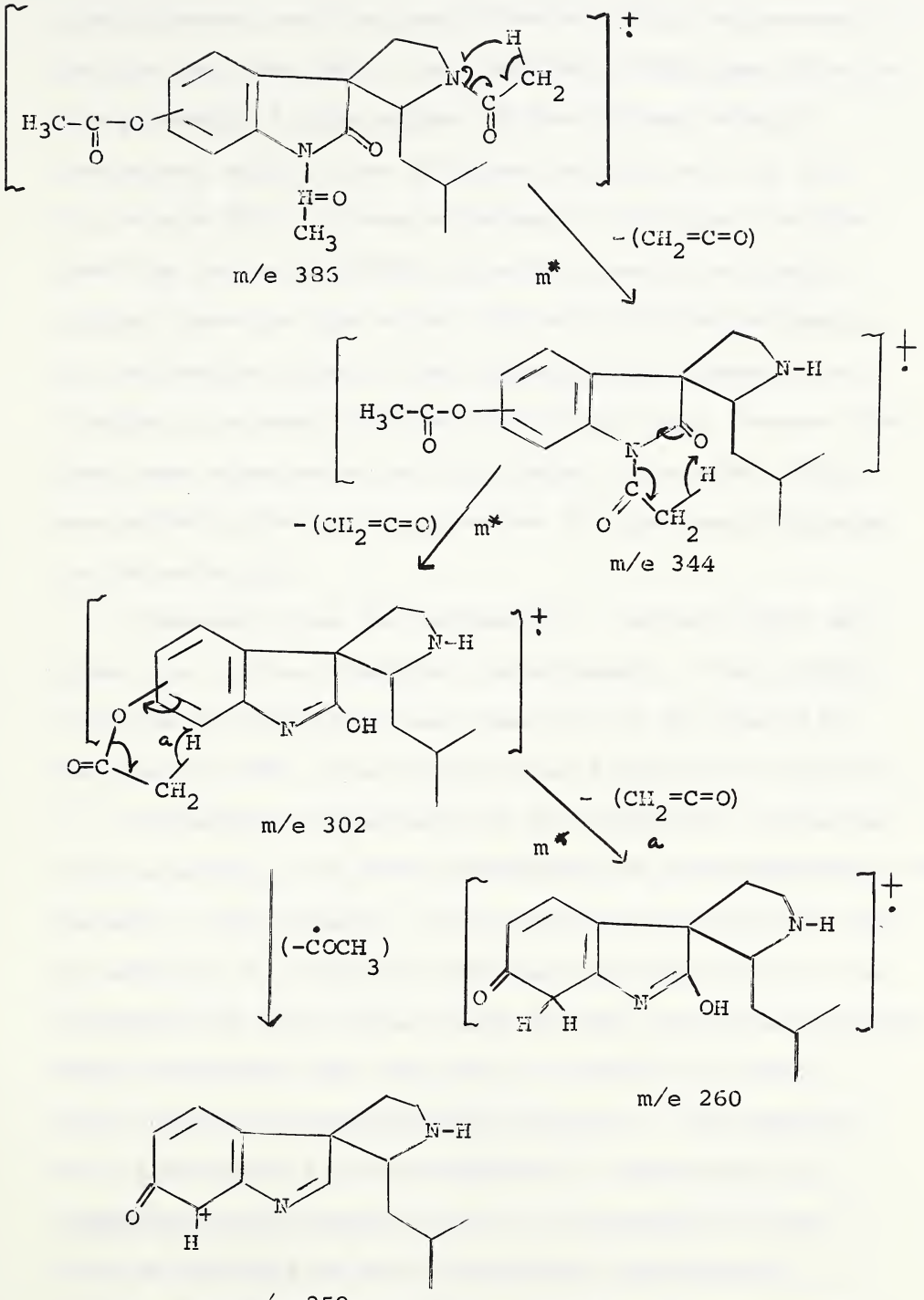
a (M-17)

Crassanine (38) is an exception, as it does not exhibit the loss of a hydroxyl group. Compound 2, which contains a hydroxyl group on the aromatic ring, showed a peak at (M-17), however it is less than 2 per cent relative abundance. The M-17 may arise from the oxindole

moiety of Compound 2 and not involve the phenolic hydroxyl group.

Further information on the structure of Compound 2 can be deduced from spectral evidence obtained from the acetylated compound. This compound was a brown oil, which could not be crystallized, however upon TLC it was shown to be a pure compound. The IR spectrum (Figure XI) in chloroform showed a CH stretching band (3010 and 2985 cm^{-1}), an ester carbonyl band (1762 cm^{-1}), a lactam carbonyl band (1720 cm^{-1}) and a broad band (1665-1590 cm^{-1}) characteristic of an amide carbonyl and a benzene ring. There was no band at 3225 cm^{-1}, which would indicate that the indolic hydrogen was replaced by an acetyl group. The NMR spectrum (Figure XIV) in chloroform showed a singlet at τ 1.97 (1 proton), τ 2.77 (1 proton) and a multiplet centered at τ 3.00 (1 proton). These three bands were due to the aromatic protons and were not exchanged upon the addition of D_2O. A three proton singlet at τ 7.32, a three proton singlet at τ 7.64 and a three proton doublet at τ 7.90 were due to the methyl groups on the two amides and the ester. A six proton multiplet at τ 9.28 was due to the two methyl groups on the isobutyl side chain.

The molecular formula, $C_{21}H_{20}N_2O_5$ (m/e 386), was established by high resolution mass spectrometry (Figure XIII). The molecular ion fragments by a transfer of hydrogen from the acetyl methyl to the N-5' nitrogen



SCHEME 5

simultaneously with a loss of ketene which may account for the ion, m/e 344. The transfer of hydrogen from the acetyl methyl to the oxygen of the lactam carbonyl concurrent with a loss of ketene may account for the ion at m/e 302. A loss of ketene by hydrogen transfer gave the ion at m/e 260, while the loss of an acetyl radical gave the ion at m/e 259 and provided evidence for an acetoxy group. The compound then fragments according to scheme 5 of the unacetylated base, however the base peak appeared at m/e 43 instead of m/e 84. This was probably due to the presence of three acetyl groups in the molecule.

Compound 2 has two asymmetric centers which may give rise to four possible stereoisomers. The optical rotation of Compound 2 was taken on a 0.1% ethanolic solution at 25°. The rotation was found to be +174.8°.

Yohimbinoid alkaloids can be oxidatively converted into oxindoles, but those reactions are stereochemically dependent. For example, cis-DE-yohimbinoid alkaloids such as uncarine B, corynoxine and caraparaubine (45) can be converted by lead tetraacetate to give acyloxyindolenines. These compounds upon refluxing in methanolic acetic acid yield the corresponding oxindoles. The presence of 1-isobutyl-1,2,3,4-tetrahydro- β -carboline in E. commutata would suggest that it is possible to also have an oxindole as the biosynthetic counterpart.

From the above data, Compound 2 was tentatively

identified as 4'-isobutyl-3,3'-spiro-pyrrolidino-oxindole with a hydroxyl group on C-5. Work is in progress on the x-ray crystallography determination of the actual structure of Compound 2 (46).

Agurell, Holmstedt and Lindgren (44) used a 5% OV-17 phenylmethylsilicone column (2.25 m x 3.2 mm i.d. glass tube) in order to separate, isolate and identify the alkaloidal components of B. rusbyana by the use of combined gas chromatography and mass spectrometry. In our attempt to separate alkaloidal compounds from the eluent fractions of Extract E.2.2, which were first run through a silicic acid column, a 3% OV-17 phenylmethyl-silicone column (1.85 m x 3.2 mm i.d. glass tube) was used. Six eluent fractions in addition to tetrahydro harmane and norharmane were passed through the column on the F and M model 700 Gas Chromatograph. The results proved to be quite satisfactory especially with the choroform-methanol 10% extract which showed four peaks. As the samples could not be collected from this instrument it was decided to use a combination of gas chromatography and mass spectrometry. A Varian Aerograph Hi-Fi 1200 gas chromatograph was connected to the MS 12 mass spectrometer. The column used on the F and M Model 700 was too large for this instrument and a smaller column was made which had an internal diameter of 1.6 mm. All attempts to isolate the pure alkaloids from the gas chromatograph and record them simultaneously

on the mass spectrometer were unsuccessful due to technical difficulties with the ion separator. Although these experiments were unsatisfactory, it is felt that the gas chromatograph - mass spectrometer combination may have great promise in this work.

After Compound 2 and the neutral materials were removed from extract E.2, the remaining material was chromatographed on a silicic acid column. The use of various solvent combinations as eluents did not result in the isolation of any pure compounds. Two compounds (R_f 0.32 and 0.42) were separated and removed from preparative TLC plates (n-butanol:acetic acid:water - 4:1:1) by the use of methanol, dried with sodium sulphate and evaporated. These compounds were visible on the plates after the solvent had moved 15 cm, and it appeared that they were oxidized in the solvent system or upon exposure to the atomosphere. Other solvent systems (hexane: chloroform:methanol - 40:20:11, benzene-chloroform - 5:1, chloroform-methanol - 9:1, and n-propanol-ammonium hydroxide - 4:1) were employed, but the separation was poor. Various solvent and solvent combinations were used unsuccessfully in an attempt to crystallize these two compounds. The compound with R_f 0.32 was dissolved in chloroform and rechromatographed on a column of alumina, activity III. Some impurities were left behind on the column, however it still could not be recrystallized. The preparation of a salt was not successful. Although

no further work was attempted on these two compounds, it would appear that the use of gas chromatography - mass spectrometry may give better results.

Extract E.3 (basic pH 11) was examined by comparing it to extract E.2 through the use of TLC. Three solvent systems were used for both silica gel G and aluminum oxide G (Table 6). There did not appear to be much difference in these two extracts, although the n-butanol:acetic acid:water - 4:1:1 solvent system revealed that there was at least one more alkaloidal spot in the E.2 extract. This comparison was done on the crude alkaloidal fraction. The removal of neutral materials and separation on an alumina or silicic acid column of Extract E.3 followed by a comparison with extract E.2.2 might give a clearer indication of the alkaloids present.

Extract E.4 (quaternary alkaloids) was examined by the use of TLC. Four solvent systems were used with the silica gel G plates and two solvent systems were used with aluminum oxide G (Table 7). The presence of only one alkaloidal substance was shown when the plates were sprayed with Dragendorff reagent. The large alkaloidal spot corresponds to the R_f of choline chloride run on the same plate. It gave the characteristic violet color of choline with Dragendorff reagent. No attempt was made to isolate the suspected choline. TLC showed that choline was less readily adsorbed by alumina oxide G than by silica gel G.

EXPERIMENTAL

Instruments, Apparatus and Materials

The following instruments were used in this study:

1. Beckman DB and DK 2 Ultraviolet Spectrophotometers.
2. Beckman IR 10 Infrared Spectrophotometer.
3. Varian Associates A.60 and H.R. 100 Nuclear Magnetic Resonance Spectrophotometers using tetramethylsilane as reference.
4. A.E.I. M.S. 9 Mass Spectrophotometer.+
5. A.E.I. M.S. 12 Mass Spectrophotometer.
6. F and M model 700 Gas Chromatograph, dual column, dual flame, ionization detector. Gas chromatographic column employed was a 3% O.V. 17 on DMCS treated Chromosorb W, regular 60-80 mesh, 1.85 m x 3.2 mm i.d. glass column.
7. Varian Aerograph Hi-Fi 1200 Gas Chromatograph.
8. Carl Zeiss Circle Polarimeter.

The apparatus used in this study were:

1. Desaga SII adjustable spreader.
2. Desaga sample applicator TNO-Delft system.
3. Hot-stage Fisher-Johns melting point apparatus and a Thomas Hoover Capillary melting point apparatus.++
4. Buchler Flash evaporator.

+ All mass spectra are quoted in terms of relative abundance, with the most intense peak ("base peak") being taken as 100%.

++ All temperatures are in degrees centrigrade and the melting points are uncorrected.

5. Wiley Mill.

6. Beckman Zeromatic II pH meter.

The following material was used in this study:

Elaeagnus commutata root bark was collected near Viking, Alberta in the summer of 1967. It was air dried before being ground in a Wiley mill.

Thin Layer Chromatography (TLC) Plates

These were prepared by spreading a slurry of Silica gel G (G. Merck and Co., Darmstadt) in distilled water on 20 x 20 cm glass plates. The plates for qualitative work were coated to a thickness of 0.2 mm, while those for preparative work were 1.00 mm thick. These were allowed to dry at room temperature for about an hour and then they were placed in an oven at 110° for thirty minutes.

Development of the coated plate, after suitable activation and application of the test material was by the "oversaturation chamber" technique (47). Various eluting solvent mixtures were used, depending on the material and type of plate used. For preparative work two solvent systems (n-hexane:chloroform:methanol - 40:20:10 and n-butanol:acetic acid:water - 4:1:1) were used.

The sample was applied to the preparative plates in a thin continuous line 1.5 cm from the bottom of the plate. The loading was 15-30 mg per 20 cm (1.0 mm), corresponding to an approproximate 1:1300 to 1:700 w/w relation between sample and medium respectively.

Following development of the plates, they were allowed to dry before the separated components were removed with a modification of the "microvacuum cleaner" method (48). The pure component was recovered from the silica gel by a micro-soxhlet technique using methanol as the menstrum or using the cold extraction method.

Spray Reagents for Thin Layer Chromatography

The plates used for qualitative and quantitative work were sprayed with Ehlich spray (8 volumes of 2% p-dimethyl-aminobenzaldehyde in 95% ethanol and 2 volumes of 6N HCl)(49), Dragendorff reagent (50), and iodoplatinate reagent (51).

Adsorption Chromatography

Silicic acid 100 mesh (Mallinchrodt Chemical Works, New York) and neutral alumina (M. Woelm, via Alupharm Chemicals, New Orleans) with an activity grade III were used in the course of this investigation. The weight of the column material used was thirty to forty times the weight of the sample to be chromatographed. In all cases, unless otherwise specified, the columns were packed by gravity in chloroform. Excess solvent was removed from the column and the sample to be chromatographed was dissolved in a minimum amount of benzene or chloroform and applied to the column. The column was then eluted with solvents in the order of increasing polarity.

Extraction of the Components of Elaeagnus commutata

The finely powdered root bark (17.5 Kg) was exhaustively

extracted with petroleum ether (30-60°) in a soxhlet extraction apparatus. The marc, after drying, was continuously extracted with 95% ethanol. The combined ethanol extract was concentrated under reduced pressure to a thick dark syrup. An equal volume of the alcoholic extract was dripped into a 2% aqueous hydrochloric acid solution under vacuum at 40° and the resultant liquor was left overnight at 0°. A brown precipitate was filtered, redissolved in alcohol and then extracted again until there was no indication of alkaloids present when Valser's or Mayer's reagents were used as alkaloidal indicators. The clear filtrate was filtered through Hyflow Super-Cel (Johns-Manville Products Corp.), placed into a continuous extractor and extracted for 24 to 48 hours with chloroform. The chloroform extract was washed with water and dried over anhydrous sodium sulphate. Extract E.1, a dark brown viscous oil (151.0 g), remained after the chloroform extract was evaporated under vacuum.

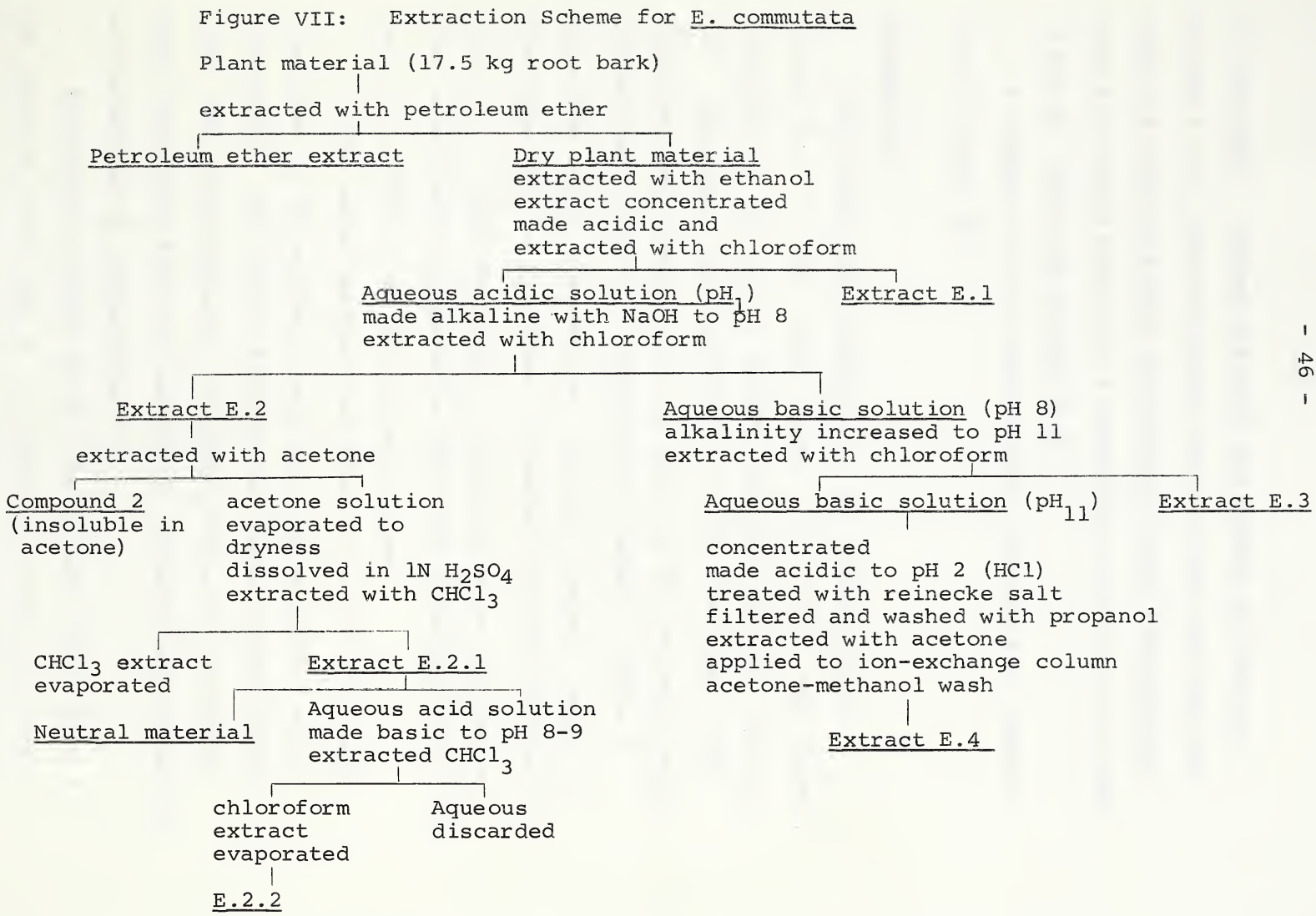
The aqueous acidic filtrate remaining after the chloroform extraction was made basic by adding cold 5N sodium hydroxide to a pH 8 (pH paper and the Beckman Zeromatic II pH meter) and then continuously extracted with chloroform. This chloroform extract upon evaporation gave a brown solid E.2 (157.0 g). Treatment of the solid E.2 with acetone dissolved all the solid except for a white solid component which subsequently was shown to be a single alkaloid (Compound 2). After Compound 2 was removed the

acetone solution containing extract E.2.1 was filtered and brought to dryness on a flash evaporator. In order to remove neutral compounds the solid extract was dissolved in 1N sulphuric acid and then extracted with chloroform. A 1N sodium hydroxide solution was added to the acidic solution until a pH of between 8 and 9 was obtained (pH paper). This was extracted with chloroform, dried, filtered and evaporated, leaving extract E.2.2.

The basicity of the aqueous solution was increased to pH 11 by the addition of a small amount of cold 5N sodium hydroxide. Since an emulsion was formed and chloroform would not pass through it, continuous extraction was not used. This basic aqueous fraction was extracted in a separatory funnel until it gave a negative Valser's test. A light brown solid E.3 (37.0 g) remained after the chloroform was taken to dryness.

The aqueous liquor was concentrated in a flash evaporator, adjusted to pH 2 with cold 10% hydrochloric acid and refrigerated for 24 hours. After separation from an insoluble residue, the dark concentrate was treated with a saturated aqueous solution of reinecke salt to completely precipitate quaternary alkaloids. After chilling 24 hours the reineckates were separated from the mixture by filtration and washed with n-propanol to remove impurities (54). The solid reineckates were dissolved in acetone and diluted with an equal volume of methanol and applied to a column of ion-exchange resin (Amberlite IRA-400 (OH^-) - 100

Figure VII: Extraction Scheme for E. commutata



to 200 mesh). Column effluent and washes of acetone-methanol (1:1) were collected until a negative alkaloid test was shown and then concentrated under reduced pressure to a solid fraction of quaternary alkaloids and bases (12.0 g). This was extract E.4.

A summary of this fractionation procedure is presented in Figure VII.

Extract E.1

Two methods were employed in the separation of extract E.1. In the first method, 5.0 g of E.1 was chromatographed on 165 g of a column made from alumina, activity III. The eluate was collected in 25 ml fractions. The fractions were combined on the basis of TLC patterns using ultraviolet light and iodoplatinate reagent. Table 1 shows the eluent composition, fraction numbers, weight of fractions and the R_f values (n-hexane:chloroform:methanol - 40:20:11). Fractions nine to fifty-six were combined and then separated by preparative TLC using n-hexane:chloroform: methanol -40:20:11 as the solvent system.

In the second method, two grams of extract E.1 were dissolved in 1N sulphuric acid and then extracted with chloroform. The acidic fraction was made slightly basic with 10% sodium carbonate and extracted with chloroform. This chloroform fraction was dried, filtered and evaporated and separated by preparative TLC.

Preparative plates 20 cm x 20 cm were coated with 1 mm layer of silica gel G. A chloroform solution of the

TABLE 1

Data for the Alumina Column of "Extract E.1"

Eluent Composition	Fraction Numbers	Weight of Fractions (mg)	TLC (Rf Values)
Benzene	1-8	15	- - - - -
Chloroform	9-30	230	0.15, 0.22, 0.37+, 0.60+
$CHCl_3$-methanol 1%	31-38	160	0.15, 0.22, 0.37+, 0.60+
$CHCl_3$-methanol 2%	39-47	121	0.15, 0.22, 0.37+, 0.60+
$CHCl_3$-methanol 4%	48-56	232	trace 0.15, 0.22, 0.37+, 0.60+
$CHCl_3$-methanol 10%	57-65	121	trace 0.15, 0.22, 0.37+
$CHCl_3$-methanol 25%	66-74	170	trace 0.15, 0.37+, 0.51
$CHCl_3$-methanol 50%	75-84	70	0.51, 0.61
Methanol	85-117	650	0.51, 0.61

+ Alkaloidal spots were detected with Iodoplatinate reagent.

extract was applied as a straight line across the plate using a Desaga SII applicator, 1.5 cm from the bottom of the plate. The plates were developed under the super-saturated chamber conditions, using the solvent n-hexane: chloroform:methanol - 40:20:11, for a solvent run of 15 cm. Compound 1 (Rf 0.37) gave a light green color when it was exposed to ultraviolet light. It was removed using a "microvacuum cleaner" and was eluted from the silica gel using methanol as the solvent. Compound 1 could not be crystallized from single solvents or a mixed solvent system. A hydrochloride salt was prepared by adding methanolic hydrogen chloride solution to a methanol solution of Compound 1. Compound 1 hydrochloride salt precipitated from methanol upon the addition of ether and was recrystallized from methanol, m.p. 257-259°.

The following are the physical characteristics of Compound 1 HCl:

UV spectrum: $\lambda_{max}^{Ethanol}$, 223 mμ, 273 mμ, 279 mμ and 289 mμ. No change was observed upon the addition of acid or base.

IR spectrum: $\nu_{max\ (nujol)}$ 3200 (indole N-H), 1590, 1450, 1375, 1303, 1235, 1215, 1185, 1155, 1000, 730 cm^{-1}. (Figure VIII).

NMR spectrum: τ -1.10, singlet (1 proton); 0.30, multiplet (2 protons); 2.75 (centered), multiplet (4 protons); 5.38, multiplet (1 proton); 6.7, multiplet (2 protons), 7.1, multiplet (2

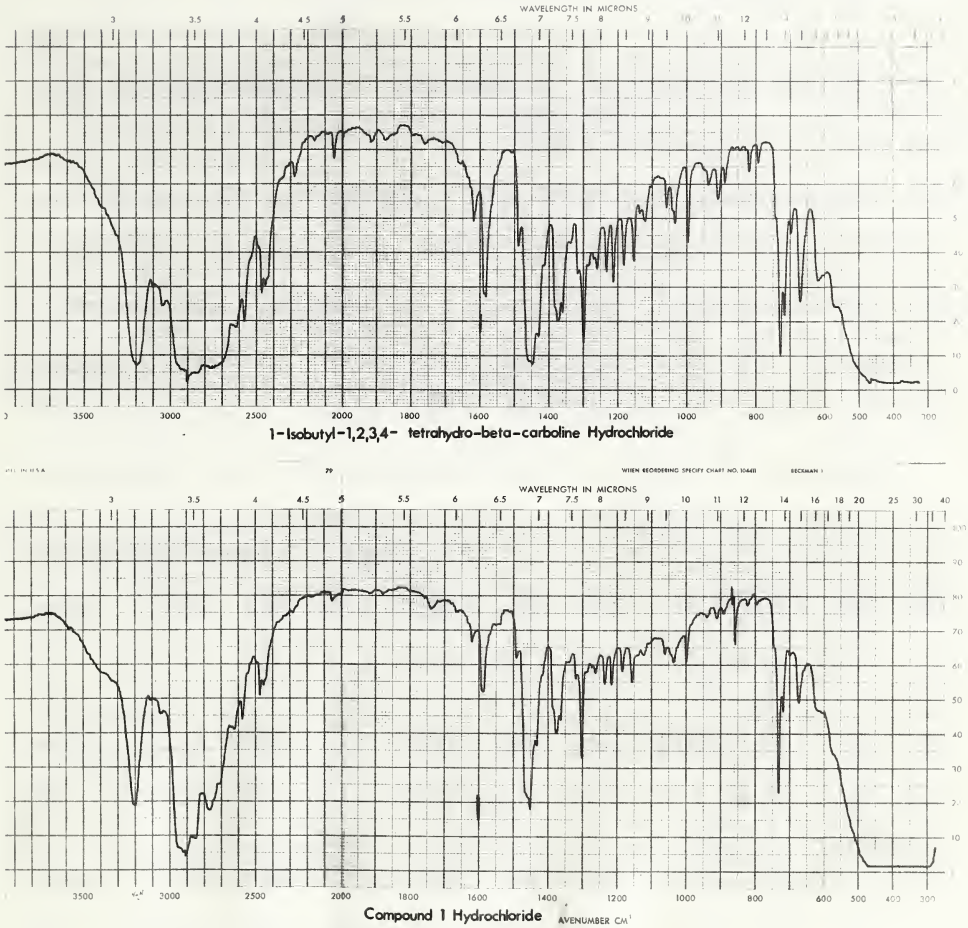


Figure VIII

Infrared Spectrum of 1-Isobutyl-1,2,3,4-tetrahydro- β -carboline HCl and Compound 1 HCl

protons), 7.95, multiplet (3 protons) 8.98, triplet (J=5 cps) (6 protons). (Figure IX).

Gas Chromatography : Compound 1 in methanol (2 µl) was injected onto a 3% O.V. 17 column installed in the F and M 700 gas chromatograph. The detector temperature, 270°; injection temperature, 290°; column temperature, 195°; carrier gas, helium; at a flow rate of 50-60 ml/min; hydrogen pressure, 15 lbs/in^2; air pressure, 25 lbs/in^2; attentuation 2, range 10^2. The retention time for this compound was 9.30 minutes from the time of injection.

Mass Spectrum: (Figure X)

m/e	229	228	227	184	172	171	170	169	156	155
%	2.0	10.6	3.5	3.2	13.8	100	4.7	6.7	8.3	3.0
m/e	154	144	143	130	129	128	118	115	86	85
%	5.4	6.3	3.2	2.2	2.6	2.9	2.2	4.3	3.0	4.7
m/e	77	38	36	29	28	27				
%	2.3	4.2	13.5	3.1	2.4	2.2				

An accurate mass determination of the molecular ion m/e 228 was made: measured : 228.1627, calculated for $C_{15}H_{20}N_2$: 228.1627.

Optical Activity: No optical activity was observed.

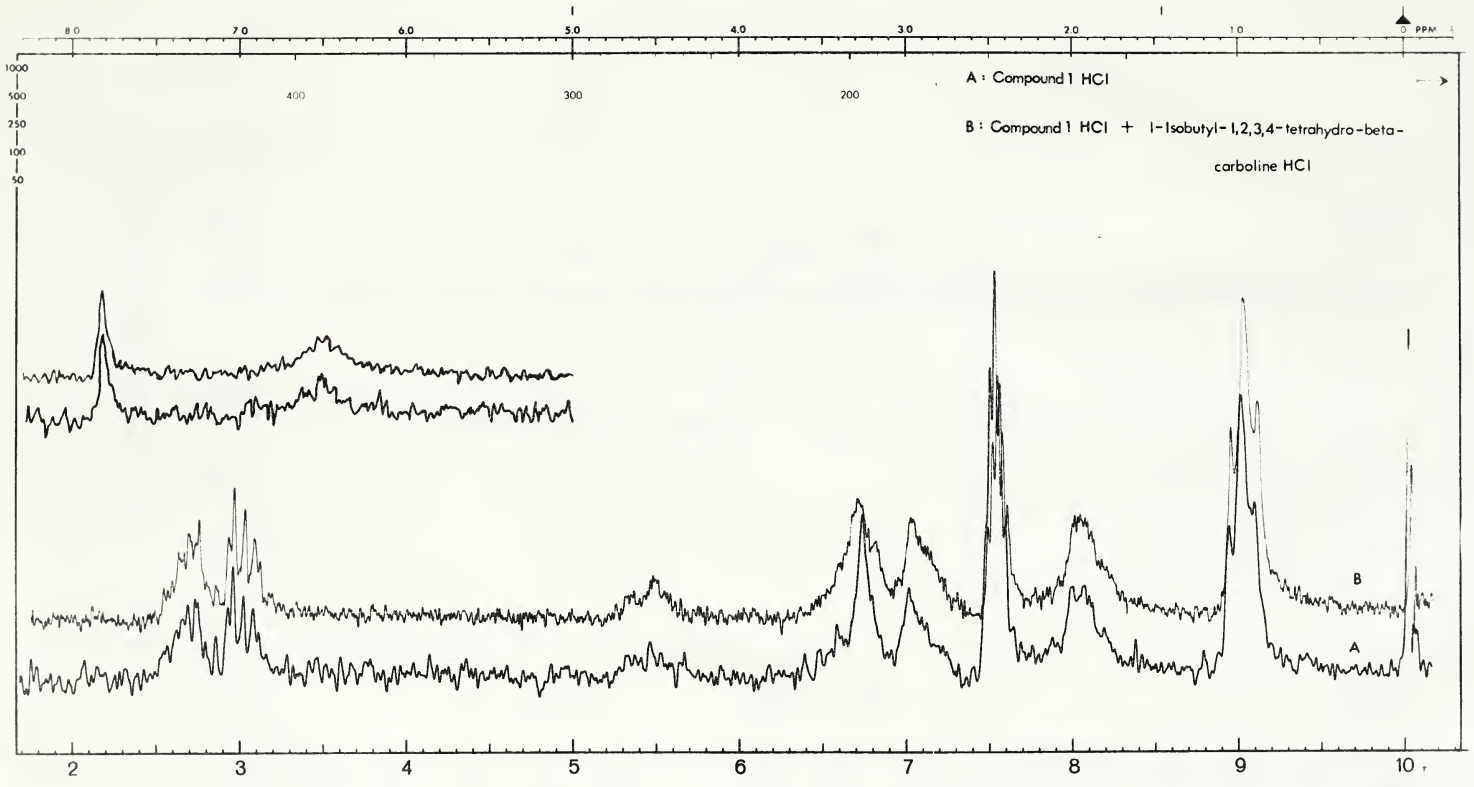


Figure IX

NMR (60 Mc) Spectrum of 1-Isobutyl-1,2,3,4-tetrahydro-β-carboline HCl and Compound 1 HCl

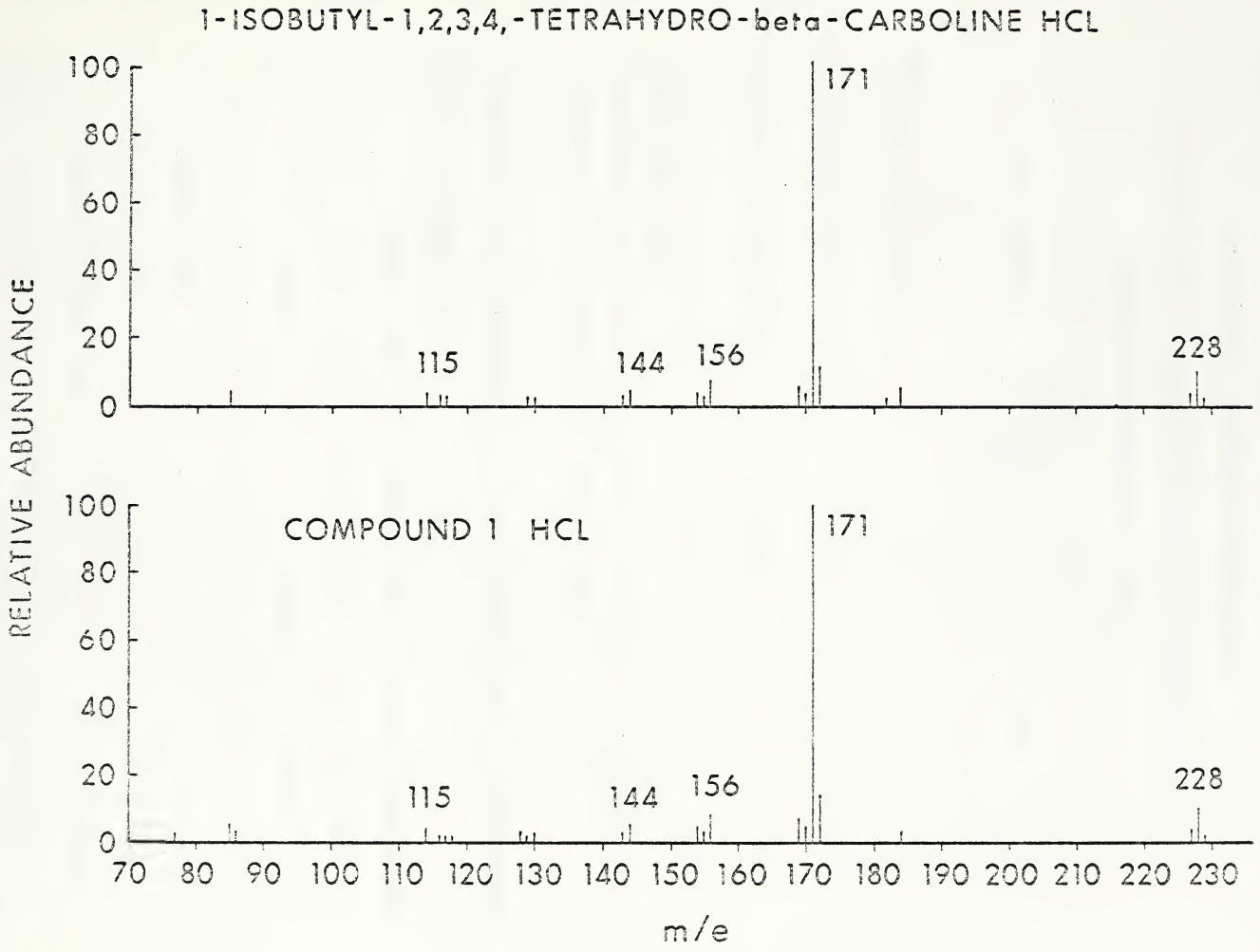


Figure X

Mass Spectrum of 1-Isobutyl-1,2,3,4-tetrahydro-
β -carboline HCl and Compound 1 HCl

TABLE 2

Thin Layer Chromatography+ of

1-Isobutyl-1,2,3,4-tetrahydro-β-carboline HCl and Compound 1 HCl

Solvent System	1-Isobutyl-1,2,3,4-tetrahydro-β-carboline HCl R_f Values	Compound HCl R_f Values
Hexane-chloroform-methanol (40:20:11)	0.37	0.37
n-Butanol-acetic acid-water (4:1:1)	0.52	0.54
Chloroform-methanol (9:1)	0.34	0.35
n-Propanol-ammonium hydroxide (8:2)	0.81	0.80

+ Plates were coated with Silica Gel G.

Synthesis of 1-Isobutyl-1,2,3,4-Tetrahydro-β-Carboline Hydrochloride Salt

An aqueous solution consisting of isovaleraldehyde (.730 g), water (20 ml), and alcohol (2 ml) was added to an ice cold aqueous solution of tryptamine hydrochloride (.540 g) to which hydrochloric acid was added to a pH 1. The mixture was stirred for half an hour at room temperature, heated to boiling for half an hour, and kept at boiling for one hour. It was cooled, made alkaline with sodium carbonate, extracted with ethyl acetate, dried, and evaporated on a flash evaporator. The residue was dissolved in methanol and a methanolic hydrogen chlor-

ide solution was added to give the salt. It was crystallized from methanol. The R_f value on TLC (Table 4), IR (Figure VIII), NMR (Figure IX) and gas chromatography retention times are identical with the salt of the natural occurring alkaloid, Compound 1.

Extract E.2

The addition of acetone to extract E.2 resulted in the precipitation of the major alkaloid, Compound 2. It was a white flakey powder which crystallized from methanol, m.p. 250-252°.

The following are the physical characteristics of Compound 2:

Elemental Analysis[+] :

Found:	C 68.74%,	H 7.76%,	N 10.93%
Calculated:	C 69.23%,	H 7.75%,	N 10.75%
Found:	CH_3O 0.55%,	CH_3N 0.61%,	CH_3C 5.55%
Calculated:	CH_3O - -	CH_3N - -	CH_3C 5.72%

Molecular formula: $C_{15}H_{20}N_2O_2$ (260)

UV spectrum[++] : $\lambda^{\text{ethanol}}_{\text{max}}$ and $\lambda^{\text{acid}}_{\text{max}}$ 223 mμ ($\log \varepsilon = 4.42$), 260-262, mμ ($\log \varepsilon = 3.58$), 284 mμ ($\log \varepsilon = 3.52$), 294 $m\mu_{inf}$ ($\log \varepsilon = 3.41$).

+ C, H and N - Microanalytical Laboratory, Chemistry Department, University of Alberta. CH_3O, CH_3N and CH_3C Dr. Franz Pascher, Mikroanalytisches Laboratorium, 53 Bonn, Buschstraße 54. Calculated for $C_{15}H_{20}N_2O_2$.

++ Log ε calculated on basis of $C_{15}H_{20}N_2O_2$ (260).

λ_{max}^{base} 210 mμ (log ε = 4.40), 220 mμ$_{inf}$ (log ε = 4.31), 300 mμ (log ε = 3.63).

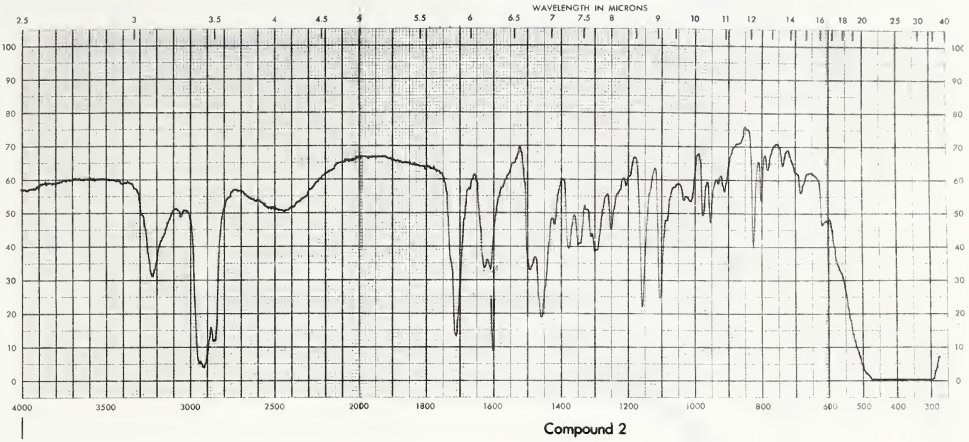
IR spectrum : $\nu_{max(nujol)}$ 3225 (N-H), 2450, 1710 (C=O of a lactam), 1630, 1610, 1380 1375, 1155, 1105, 825 cm^{-1}. (Figure XI).

$\nu_{max(KBr)}$ 3420, 3225, 2450, 1710 1670, 1630, 1495, 1160, 1105, 825 cm^{-1}.

NMR spectrum : τ -0.02, singlet (1 proton); 3.01, doublet (one proton, J=8 cps); 3.69, doublet (two aromatic protons); 6.92, doublet (two aromatic protons); 6.92, (centered), multiplet; 7.98, triplet (2 protons); 9.27, triplet (J=7 cps) (6 protons). (Figure XII).

Mass Spectrum (Figure XIII)

m/e	261	260	245	203	176	175	174	163
%	8.8	49.1	5.7	3.2	2.9	12.4	4.7	3.9
m/e	162	148	146	133	102	99	98	86
%	11.0	3.5	7.5	3.4	5.1	19.5	7.8	3.1
m/e	85	84	77	70	68	57	56	51
%	6.9	100.0	3.5	3.2	4.2	23.4	86.5	2.7



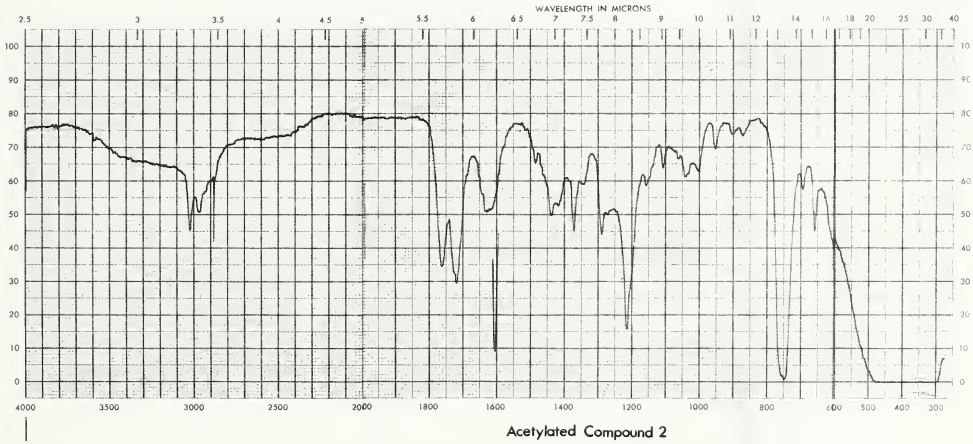


Figure XI

Infrared Spectrum of Compound 2

and Acetylated Compound 2

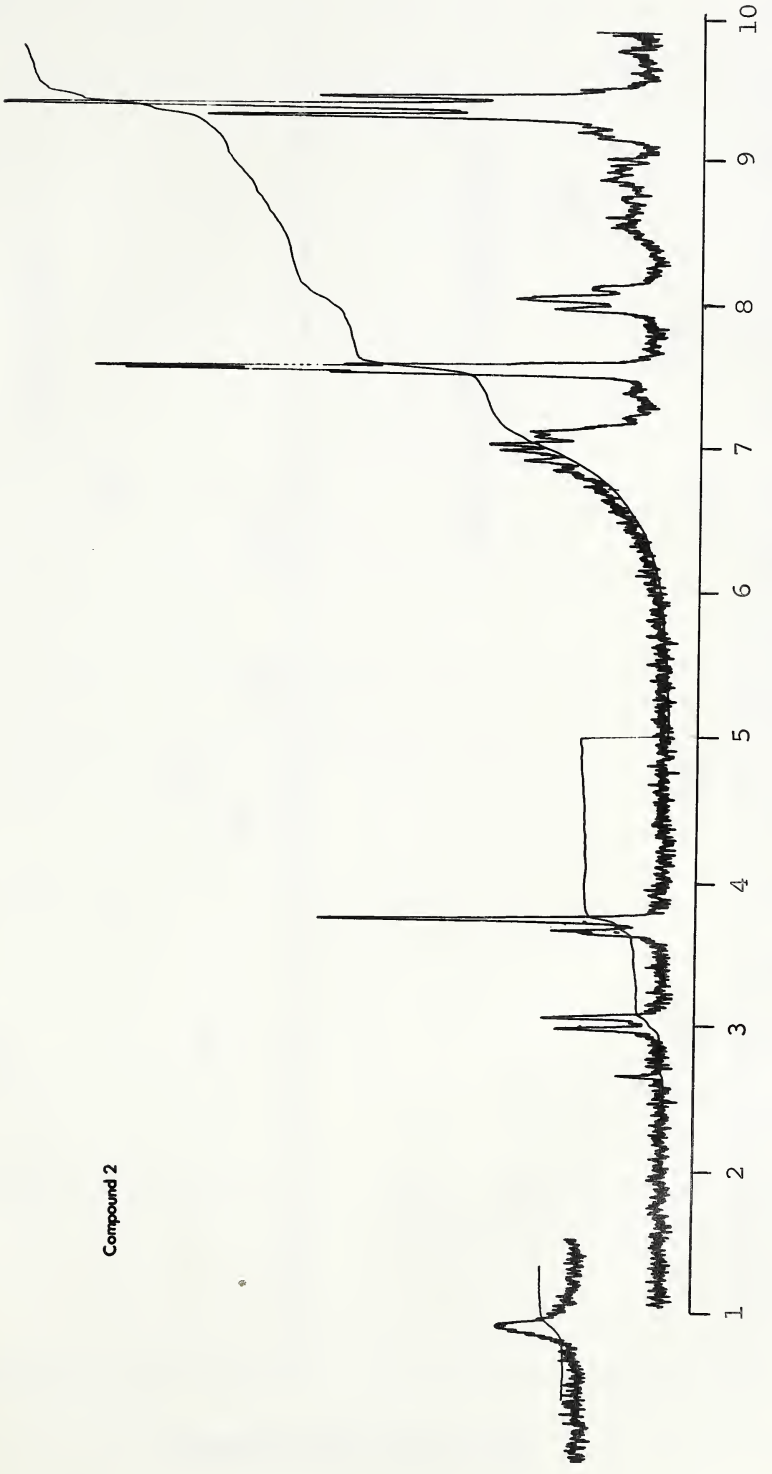


Figure XII

NMR (100 Mc) Spectrum of Compound 2

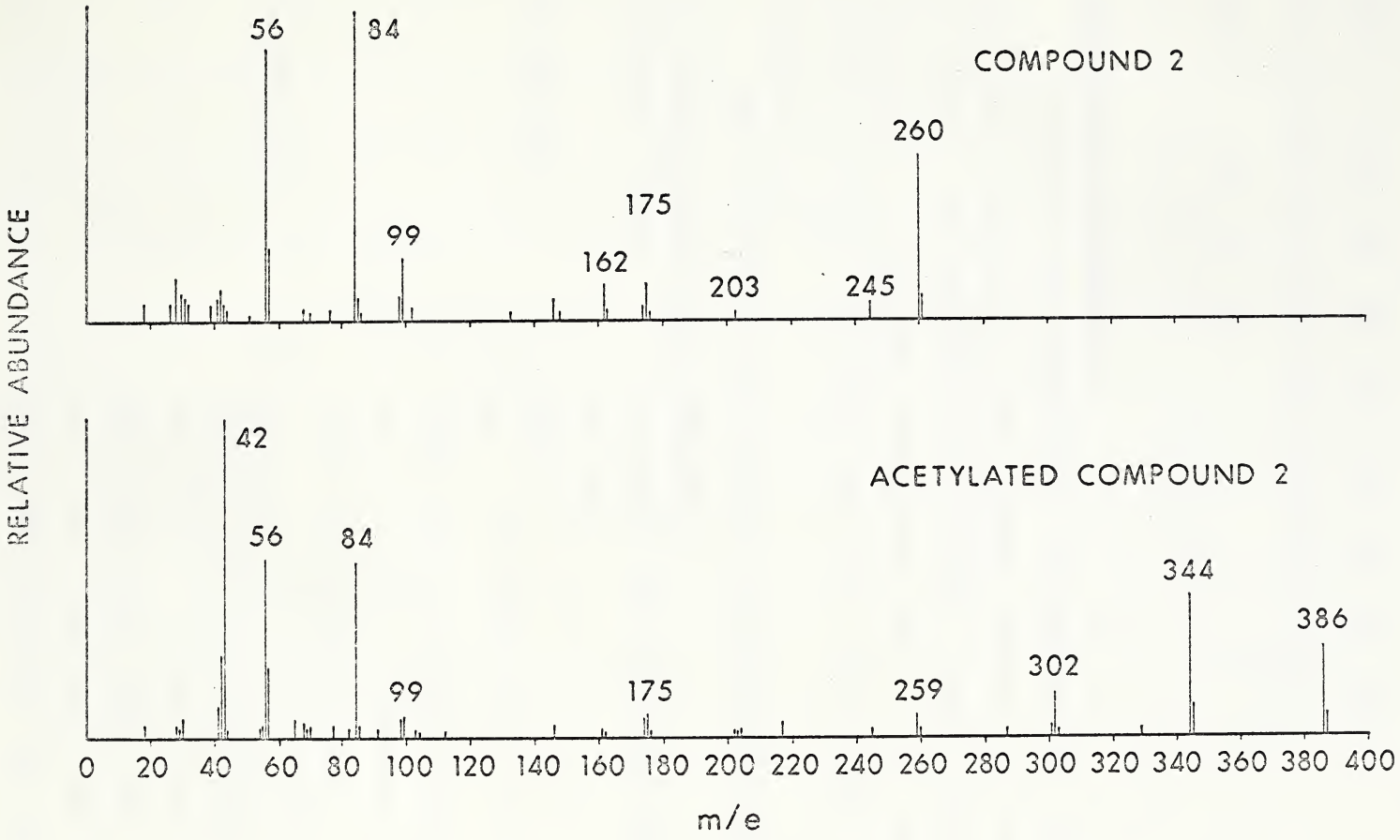


Figure XIII

Mass Spectra of Compound 2 and Acetylated Compound 2

m/e	44	43	42	41	39	32	31	30
%	4.6	6.1	10.1	7.8	4.6	5.3	7.0	9.3

m/e	28	27	18
%	13.9	6.3	6.1

Acetylation of Compound 2

Compound 2 was dissolved in one part pyridine, one part acetic anhydride and the reaction mixture was allowed to stand at room temperature for 12 hours. Excess solvent was removed by azeotropic distillation with toluene under reduced pressure. The acetyl derivative could not be recrystallized.

The following are the physical characteristics of the acetyl derivative of Compound 2.

Molecular formula : $C_{21}H_{26}N_2O_5$ (386) from mass spectral data.

UV spectrum : $\lambda^{\text{ethanol}}_{\text{max}}$ 205 $m\mu_{inf}$ (log ε = 4.51), 275 $m\mu$ (log ε = 3.13), 285 $m\mu_{inf}$ (log ε = 3.03). No change when sodium hydroxide was added.

IR spectrum : $\nu_{max(CHCl_3)}$ 3010, 2985 (CH stretching), 1762 (C=O of an ester), 1720 (C=O of a lactam), broad band (1665-1590), 1490, 1440, 1415, 1375, 1290, 1215, 1160, 1105, 1045, 1000, 955, 900, 870, 750 cm^{-1}. (Figure XI).

NMR spectrum : τ 1.97, singlet (1 proton); 2.77, singlet (1 proton); 3.00 (centered) multiplet (1 proton); 6.20 (centered), multiplet (2 protons); 7.32, singlet (3 protons); 7.64, singlet (3 protons); 7.90, doublet (3 protons); 8.70, singlet (2 protons), 9.28, multiplet (6 protons). (Figure XIV).

Mass Spectrum: (Figure XIII)

m/e	387	386	345	344	329	303	302	301
%	6.5	26.0	9.5	44.0	3.3	2.7	14.0	4.3
m/e	287	260	259	245	217	204	203	202
%	3.2	2.2	7.7	2.4	4.8	2.7	2.8	2.8
m/e	176	175	174	162	161	146	112	104
%	2.0	7.5	6.1	2.1	2.7	4.0	2.1	2.1
m/e	103	99	98	91	85	84	82	77
%	2.3	7.1	6.7	3.1	3.7	55.3	3.2	3.9
m/e	70	69	68	65	57	56	55	54
%	3.3	3.2	5.1	5.1	22.7	56.7	3.8	3.2
m/e	44	43	42	41	30	29	28	18
%	3.5	100.0	25.3	9.5	5.4	2.1	3.8	3.9

An accurate mass determination of the molecular ion m/e 228 was made : measured : 386.1848, calculated : 386.1842.

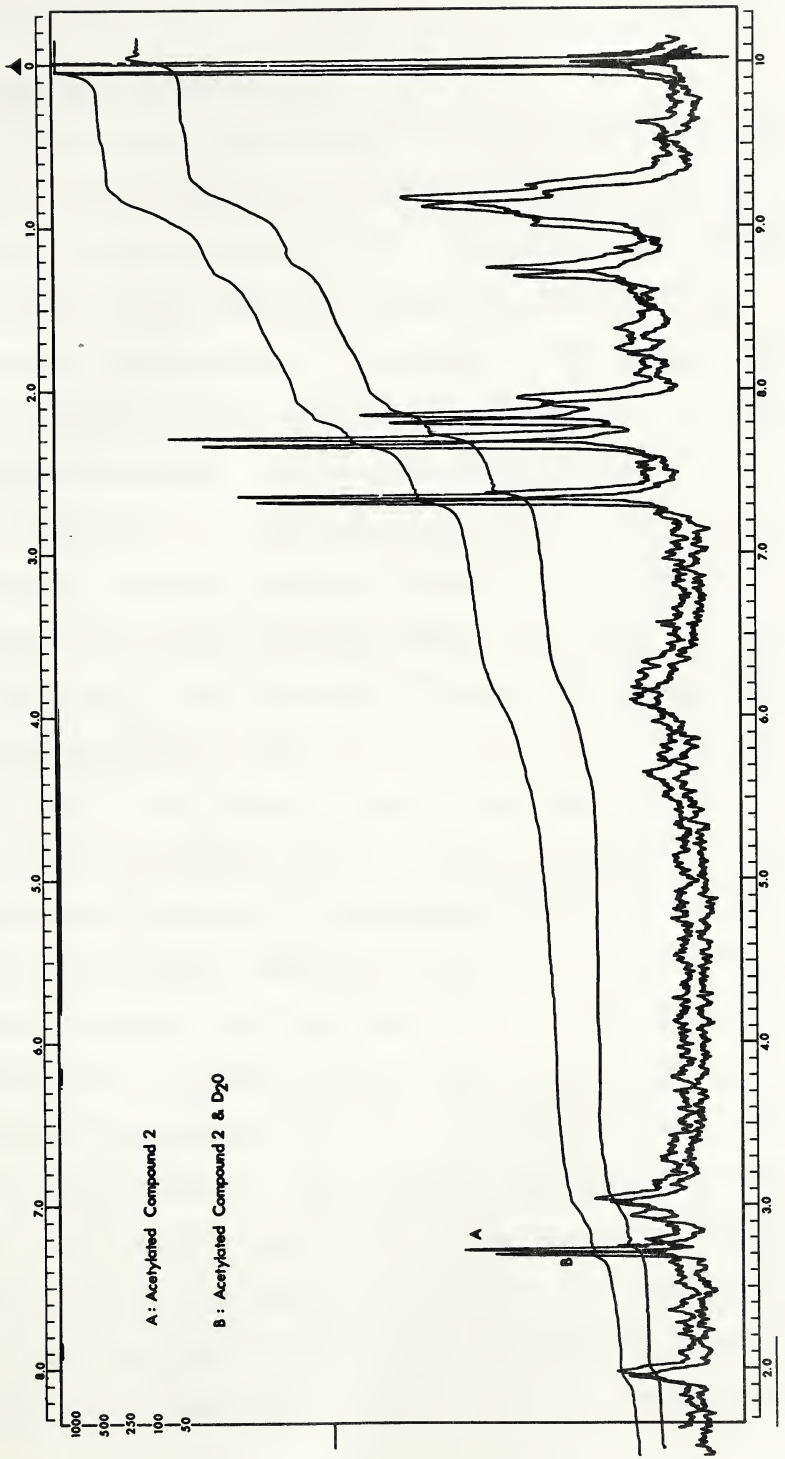


Figure XIV

NMR (60 Mc) Spectrum of Acetylated Compound 2

Optical Rotation: $[\alpha]_D^{25}$ + 174.8 (c 0.1, EtOH)

Extract E.2.1 and E.2.2

The removal of Compound 2 from extract E.2 gave extract E.2.1. Eight grams were chromatographed on 320 g of an alumina column, activity III. Fractions were combined on the basis of TLC patterns using ultraviolet light, iodoplatinate or Dragendorff's reagent. The eluent composition, weight of fractions and the Rf values (n-butanol: acetic acid:water - 4:1:1) are shown in Table 3.

Extract E.2.2 was obtained from E.2 by removing Compound 2 and any neutral material that might be present. It was hoped that a better separation could be obtained by employing a silicic acid column. Six grams were chromatographed on 256 g of silicic acid. Fractions were combined on the basis of the TLC pattern as previously reported for extract E.2.1. Table 4 showed the eluent composition, weight of fractions and the Rf values.

As no single compounds could be isolated from the silicic column, the fractions which showed positive Dragendorff or iodoplatinate spots were combined and separated by preparative TLC. Various solvent systems were used, however, only n-butanol-acetic acid-water (4:1:1) gave any suitable separation. Two compounds, which were visible after the solvent run of 15 cm, with R_f values of 0.32 and 0.42 were removed from the plates, however pure crystalline compounds or salts could not be obtained.

TABLE 3

Data for the Alumina Column of "Extract E.2.1"

Eluent Composition	Weight of Fractions (mg)	TLC (R_f Values)
Chloroform	100	0.42, 0.46+, 0.60, 0.69
$CHCl_3$-methanol 2%	170	0.36+, 0.40, 0.46+, 0.60, 0.79
$CHCl_3$-methanol 4%	730	0.27, 0.36+, 0.46+, 0.50, 0.60
$CHCl_3$-methanol 10%	300	0.31, 0.37+, 0.48+, 0.62
$CHCl_3$-methanol 25%	330	0.49, 0.57, 0.62, 0.66
$CHCl_3$-methanol 50%	550	0.29, 0.35, 0.0.40, 0.47, 0.51, 0.60
Methanol	690	0.29, 0.35, 0.51, 0.61

+ Alkaloidal spots were detected with Dragendorff reagent.

TABLE 4

Data for the Silicic Acid Column of "Extract E.2.2"

Eluent Composition	Weight of Fractions (mg)	TLC (Rf Values)
Chloroform	170	0.52, 0.89
$CHCl_3$-methanol 1%	160	0.40, 0.56, 0.62, 0.70, 0.89
$CHCl_3$-methanol 2%	235	yellow fl.(0-.20), 0.31, 0.45, 0.52, 0.60
$CHCl_3$-methanol 4%	350	yellow fl.(0-.15), 0.26, 0.45, 0.52, 0.60
$CHCl_3$-methanol 10%	230	0.27, 0.32+, 0.36, 0.42+, 0.50, 0.56
$CHCl_3$-methanol 25%	530	0.27, 0.32+, 0.36, 0.42+, 0.50, 0.56
$CHCl_3$-methanol 50%	345	0.27, 0.32+, 0.36, 0.53
$CHCl_3$-methanol 75%	256	0.27, 0.31, 0.47
Methanol	2015	0.28, 0.37, 0.47, 0.54

+ Alkaloidal spots were detected with Dragendorff or Iodoplatinate reagent

Gas Chromatography of Fractions from Silicic Acid Column:

Various eluent fractions from the silicic acid column of extract E.2.2 were dissolved in methanol and subjected to gas chromatography. Two µl samples of 5 fractions were injected onto a 3% O.V. 17 on DMCS treated Chromosorb W, regular 60-80 mesh, 1.85 m x 3.2 mm i.d. glass column under the following conditions:

Detector temperature:	270°
Injection temperature:	290°
Column temperature:	195°
Carrier gas:	Helium
Carrier gas flow:	50-60 ml/min
Hydrogen pressure:	15 lbs/in^2
Air pressure:	25 lbs/in^2
Attenuation:	2 to 50
Range:	1 to 10^2

TABLE 5

Gas Chromatography+ of Fractions from Extract E.2.2

Column Eluent	Number of Peaks	Retention Times (in mins) (from time of injection)
$CHCl_3$-methanol 1%	none	- - -
$CHCl_3$-methanol 10%	4	6.20, 8.25, 9.66, 10.50
$CHCl_3$-methanol 25%	1	5.20
$CHCl_3$-methanol 50%	1	5.20
$CHCl_3$-methanol 75%	1	5.20
Methanol	1	6.33

+ Two reference alkaloids 1,2,3,4-tetrahydroharmane and norharmane had retention times of 5.15 and 7.90 minutes respectively from time of injection. 1-isobutyl-1,2,3,4-tetrahydroharmane had a retention time of 9.30 minutes from time of injection.

Extract E.3

Extract E.3, which is very similar to extract E.2 was not separated into various fractions. A comparison of the two extracts by means of various TLC systems revealed that they were very similar (Table 6).

Extract E.4

The quaternary extract (E.4) which was separated by the use of TLC is presented in Table 7.

NMR Data for Model Compounds

The use of various model compounds such as the 1-alkyl-1,2,3,4-tetrahydro-β-carbolines, 5-hydroxyindole and 5-hydroxyoxindole are a useful aid in determining the structure of the isolated alkaloids. The NMR data is found in Table 8.

TABLE 6

Thin Layer Chromatography of "Extract E.2 and E.3"

Adsorbent	Solvent System	R_f Values for E.2	R_f Values for E.3
Silica Gel G	chloroform-methanol (1:1)	0.41, 0.47+, 0.63+	0.41, 0.47+, 0.63+
Aluminum Oxide G	chloroform-methanol (1:1)	0.00+, 0.90	0.00+, 0.90
Silica Gel G	n-propanol-ammonium hydroxide (4:1)	0.30, 0.60+, 0.66+	0.30, 0.60+, 0.66+
Aluminum Oxide G	n-propanol-ammonium hydroxide (4:1)	0.49, 0.77+, 0.84+	0.49, 0.77+, 0.84+
Silica Gel G	n-butanol-acetic acid-water (4:1:1)	0.25, 0.28+, 0.35+, 0.45, 0.48	0.25, 0.35+ 0.45, 0.48
Aluminum Oxide G	n-butanol-acetic acid-water (4:1:1)	streak, 0.89+	streak, 0.89+
Silica Gel G	n-hexane-chloroform-methanol (40:20:11)	0.00+	0.00+

+ Alkaloidal spots were detected with Dragendorff Reagent.

TABLE 7

Thin Layer Chromatography of "Extract E.4"

Adsorbent	Solvent Systems	R_f Values
Silica Gel G	chloroform-methanol (1:1)	0.00+, 0.12, 0.45
Silica Gel G	n-butanol-acetic acid-water (4:1:1)	0.04+, 0.06, 0.25, 0.34, 0.42
Silica Gel G	n-propanol-ammonium hydroxide (4:1)	0.00+, 0.16, 0.23
Silica Gel G	n-hexane-chloroform-methanol (40:20:11)	No movement
Aluminum Oxide G	n-butanol-acetic acid-water (4:1:1)	0.78+, 0.80
Aluminum Oxide G	Chloroform-methanol (1:1)	0.00, 0.81+, 0.89

+ Alkaloidal spot corresponds to the R_f of choline chloride run on the same plate. It has the largest spot area and characteristic violet color of choline reacted with Dragendorff reagent.

TABLE 8

NMR Data of Model Compounds

Compound	NMR Spectrum (τ)
1-ethyl-1,2,3,4-tetrahydro-β-carboline HCl	-0.10+, broad multiplet (1 proton); 0.40+, broad multiplet (1 proton); 2.80, multiplet (4 protons); 5.45, multiplet (1 proton); 6.25; singlet (1 proton); 6.65, multiplet (2 protons); 7.0, multiplet (2 protons); 7.85, multiplet (2 protons); 8.90, triplet (3 protons).
1-isobutyl-1,2,3,4-tetrahydro-β-carboline HCl	-1.10+, singlet (1 proton); -0.10+, broad multiplet (1 proton); 1.0++, broad multiplet (1 proton); 2.80, multiplet (4 protons); 5.45, singlet (1 proton); 6.68, singlet (2 protons); 6.98, singlet (2 protons); 8.92, doublet (2 protons); 9.09, doublet (2 protons).
1-n-butyl-1,2,3,4-tetrahydro-β-carboline HCl	-1.11+, singlet (1 proton); 0.25+ broad multiplet (2 protons); 2.85, multiplet (4 protons); 5.40; singlet (1 proton); 6.75, multiplet (2 protons); 7.00, singlet (2 protons); 7.90, multiplet (2 protons); 8.55, multiplet (4 protons); 0.05, multiplet (3 protons).
Methyl ester of tetrahydronorharmane carboxylic acid	0.17+, singlet (1 proton); 2.90, multiplet (4 protons); 6.08, singlet (2 protons); 6.25, singlet (3 protons); 7.05, multiplet (2 protons); 7.75+, singlet (1 proton).
5-hydroxyindole	-0.70, singlet (1 proton); 2.80, multiplet (2 protons); 3.10, doublet (1 proton); 3.35 (centered), two doublets (1 proton); 3.80, triplet (1 proton).
5-hydroxyoxindole	0.00, singlet (1 proton); 3.25, multiplet (3 protons); 6.25, singlet (1 proton); 6.65, singlet (2 protons).

+ Disappears with D_2O

SUMMARY

Elaeagnus commutata has been examined for the presence of alkaloids. TLC of all extracts and gas chromatography of extract E.2.2 revealed the presence of at least five or six alkaloids.

The extraction of 17.5 kg of E. commutata yielded 151.0 g of an acidic fraction. Preparative TLC resulted in the isolation of an alkaloid, Compound 1. A crystalline hydrochloride salt of the alkaloid (mp 257-259°) was obtained by adding methanolic HCl to a methanol solution containing the alkaloid. Compound 1 was identified as 1-isobutyl-1,2,3,4-tetrahydro-β-carboline HCl by mixed melting point, comparison of its retention time upon gas chromatography, R_f value on TLC, NMR and IR spectra. These properties were identical for the synthetic compound and the naturally occurring alkaloid.

The addition of acetone to extract E.2 resulted in the precipitation of the major alkaloid, Compound 2. The exact structure of this alkaloid is not known, however evidence has been presented indicating that its structure may be 4'-isobutyl-3,3' -spiro-pyrrolidino-oxindole with a hydroxyl group on C-5. The ultraviolet spectrum did not give conclusive evidence for an oxindole structure, but it was useful to indicate that the alkaloid has a phenolic hydroxyl group. The presence of an indolic N-H group and a lactam carbonyl were shown by

the IR spectrum. From NMR spectra it appears that Compound 2 contained an isobutyl side chain as well as aromatic protons. The mass spectral fragmentation pattern of Compound 2 gives evidence that it is an oxindole since there may be a homolytic rupture of the 3'-4' and 6'-7' bonds yielding a molecule and an alicyclic radical. Fragments containing the indole moiety at m/e 146, 160-162 and 175 are present in Compound 2. Upon acetylation a triacetyl compound was formed, which would indicate the presence of three replaceable hydrogens on the structure of Compound 2. X-ray crystallography which is presently in progress should determine the absolute structure.

After Compound 2 and the neutral materials were removed from extract E.2, the remaining material was chromatographed on a silicic acid column. Two compounds were separated by preparative TLC, but no crystalline compounds could be obtained. No further work was attempted on these two compounds, however it would appear that the use of gas chromatography-mass spectrometry might give pure compounds.

Extract E.3 was examined by comparing it to extract E.2 through the use of TLC. Both fractions were shown to be the same, although the solvent system of n-butanol:acetic acid:water - 4:1:1 revealed the presence of at least one more alkaloidal spot in the E.2 extract.

Alkaloidal material remaining after the removal of extract E.3 was precipitated as the reineckate salt. TLC of this quaternary alkaloid fraction showed that the major component was choline.

PART II

MASS SPECTRAL STUDIES OF SELECTED beta-CARBOLINES

INTRODUCTION AND STATEMENT OF PROBLEM

In the isolation, characterization and identification of an alkaloid (1-isobutyl-1,2,3,4-tetrahydro- β -carboline) from E. commutata (see Part I) (55), it was found that a more thorough knowledge of the fragmentation pathway of various 1,2,3,4-tetrahydro- β -carbolines was required. It was felt that the synthesis and mass spectra of various 1,2,3,4-tetrahydro- β -carbolines would be useful in this study.

Since 1919, when harmaline, harmine and harmol were the only naturally occurring carboline alkaloids known, at least fifteen genera have been found to contain 1,2,3,4-tetrahydro- β -carboline, 3,4-dihydro- β -carboline and β -carboline alkaloids. Although the more complex tetrahydro- β -carbolines such as picraline, yohimbine and ajmalicine have had their mass spectral fragmentation pathways recorded and interpreted, very few of the simpler β -carbolines have been investigated.

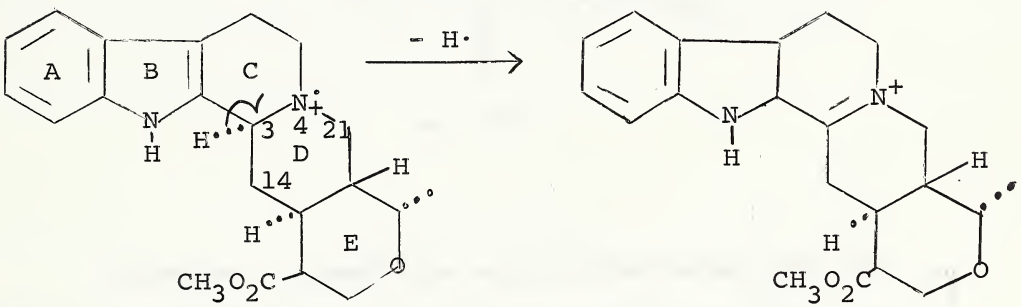
Metastable peaks and accurate mass determinations in the spectra of all the compounds studied were used in the interpretation of the fragmentation processes. Deuterium labeling techniques were also employed in the analysis of the fragmentation of 1-isobutyl-1,2,3,4-tetrahydro- β -carboline, norharmane and harmine.

LITERATURE SURVEY

The development of the use of mass spectrometry in organic chemistry and natural products in the last few years has been outstanding. Almost all common and complex functional groups have been examined. Through the use of high resolution mass measurements and isotopic labeling techniques many organic structures have been either confirmed or revised. Budzikiewicz, Djerassi and Williams (56) stated that mass spectrometry is generally employed in natural products for solving one or more of the following problems: location of functional groups and substituents, establishment of molecular weights and of empirical formulae, determination of the overall skeleton, and the elucidation of precise structure and certain stereochemical features.

A useful technique in analyzing fragmentation patterns is the introduction of a heavy atom into the compound. Deuterium labeling has been widely used in the structural elucidation of compounds into which deuterium can be introduced specifically by a reaction of known mechanism. Replacement of a specific hydrogen atom by deuterium not only labels a given portion of the molecule but also provides information on the occurrence and origin of hydrogen-transfer reactions. Budzikiewicz, Djerassi and Williams (57) have discussed methods of introducing heavy atom labels and of analyzing the mass spectral fragmentation pathways.

The mass spectra of tetra- and pentacyclic tetrahydro-β-carbolines have been investigated (43). It has been shown through extensive deuterium labeling that alkaloids such as ajmalicine or yohimbine follow characteristic decomposition paths. A pronounced (M-1) peak is shown to be predominately due to the loss of the hydrogen attached to C-3 with formation of a molecule a, in which the positive charge is stabilized by conjugation with the indole system as well as by participation of the electron pair on the N-2 nitrogen.

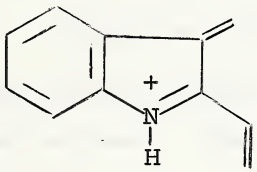


ajmalicine

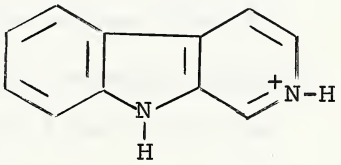
a (M-1)

The major ions in the spectra of ajmalicine and yohimbine (m/e 156, 169, 170 and 184) embodied the indole portion of the alkaloid. The m/e 156 ion was formed by a retro-Diels-Alder fragmentation of ring C, followed by a homolytic fission of the allylically activated 14-15 bond.

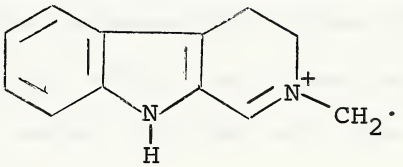
Further cleavage of the 4-21 linkage would lead to the ionized dihydro- β -carboline (m/e 170), while expulsion of an additional hydrogen atom offered a route to m/e 169. If instead of the 4-21 linkage, the 20-21 bond was broken, the ion radical (m/e 184) would be generated.



m/e 156

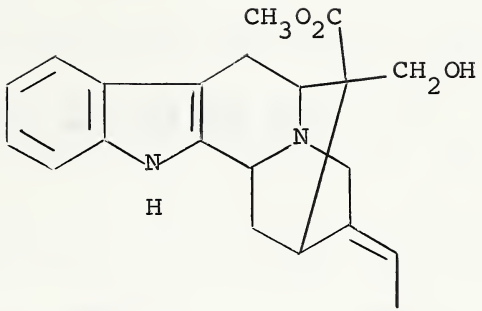


m/e 169



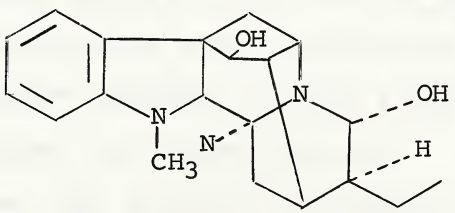
m/e 184

Sarpagine, akuammidine and related alkaloids (43) have a pronounced $(M-1)^+$ ion, which was due to the loss of the hydrogen from C-3 or C-6. The β -carboline fragments occurred at m/e 168 and 169 compared to m/e 169 and 170 for ajmalicine or yohimbine. This is due to the presence of an additional bond which has to be cleaved in ring C before the β -carboline ions at m/e 168 and 169 can be produced.



Akuammidine

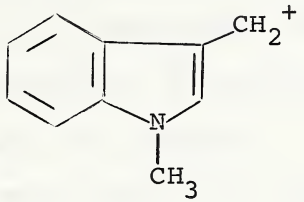
Ajmaline (58) represents a bridge between the dihydroindole alkaloids and the more fully aromatic indole derivatives. The former undergo mainly fission of the carbocyclic ring adjacent to the dihydroindole system and thus lead to fragments representing either the alicyclic or the aromatic portion, while the latter have the tendency to retain the second nitrogen with the indole part to give β -carboline ions.



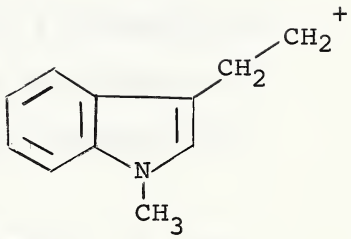
Ajmaline

The mass spectrum of ajmaline is characterized by the peaks at m/e 144 and 158, representing part of the tryptamine portion of the molecule, as well as 9-methyl-

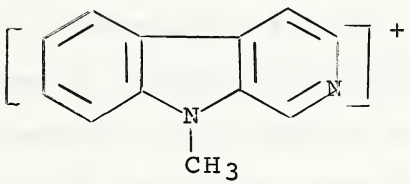
- β-carboline ions at m/e 182 and 183.



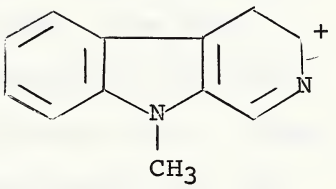
m/e 144



m/e 158



m/e 182



m/e 183

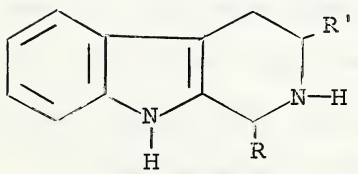
Although the mass spectra of the more complex tetrahydro- β -carbolines have been recorded and interpreted, the fragmentation pathways of only a few simpler tetrahydro- β -carbolines have been described. Agurell, Holmstedt and Lindgren (44) proposed a fragmentation mechanism for 2-methyl-1,2,3,4-tetrahydro- β -carboline, which was isolated from Banisteriopsis rusbyana. The prominent peaks of the mass spectrum were m/e 143 (base peak) and m/e 128. There was a loss of 43 and 58 mass units respectively from the molecular ion (m/e 186). The proposed mechanism would involve a retro-Diels-Alder frag-

mentation with expulsion of 43 mass units to yield a fragment m/e 143 with the ability to rearrange, loosing one methyl group and yielding a fragment m/e 128. Agurell, Holmstedt and Lindgren (59,60) isolated, 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline, 1,2-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline and 1-methyl-7-1,2,3,4-tetrahydro-β-carboline, however fragmentation pathways were not proposed.

The mass spectra of only a few aromatic β-carbolines have been studied. Agurell, Holmstedt and Lindgren (60) isolated 1-methyl-7-methoxy-β-carboline (Harmine) but no fragmentation pathway was proposed. The mass spectrum of 1-methyl-β-carboline 3-carboxylic acid has been examined by Antonaccia and Budzikiewicz (61). Three 1-alkyl-β-carbolines have been investigated (62, 63). It was shown that if the side chain is longer than two carbon atoms, the McLafferty rearrangement was the most important degradation process. One of the compounds investigated was 1-isopropyl-β-carboline, which had its most intense peak at m/e 195 corresponding to the loss of a methyl group from a carbon attached to the aromatic system. A peak at m/e 182 is formed by the loss of hydrogen radical followed by the loss of HCN from the molecular ion.

DISCUSSION

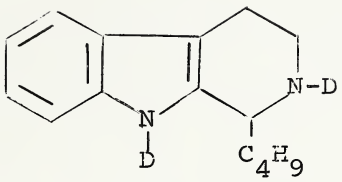
The mass spectral fragmentation for six 1-alkyl-1,2,3,4-tetrahydro- β -carbolines (Ia to If, Figure I) can be shown to follow three distinct pathways. An expulsion of an alkyl radical, to give the base peak, followed by subsequent fragmentation is the major pathway. A rearrangement of the molecule followed by the loss of a RNH radical is the second pathway. A retro-Diels-Alder fragmentation, the third pathway, is involved only with the 1-methyl-1,2,3,4-tetrahydro- β -carboline (Ia), the acid (Ig) and the ester (Ih).



I

	R	R'
a)	CH_3	H
b)	CH_3CH_2	H
c)	$CH_3CH_2CH_2$	H
d)	$(CH_3)_2CH$	H
e)	$CH_3(CH_2)_3$	H
f)	$(CH_3)_2CHCH_2$	H
g)	CH_3	COOH
h)	CH_3	$COOCH_3$

1,2,3,4-tetrahydro- β -carbolines

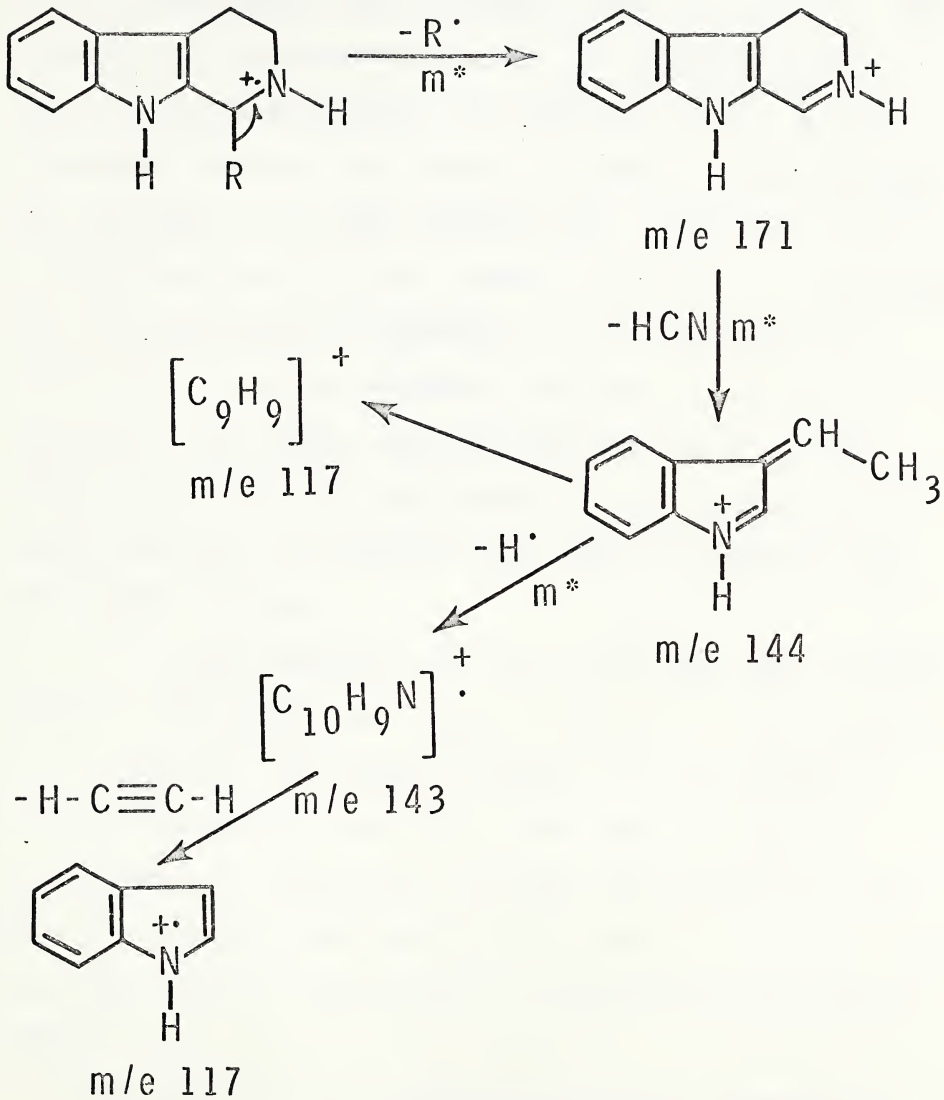


V

Deuterated 1-isobutyl-1,2,3,4-tetrahydro-β-carbolines

The base peak of simple tetrahydroisoquinolines (64) is formed by the loss of the side chain from the carbon atom adjacent to the nitrogen atom. The major fragmentation pathway of all six 1,2,3,4-tetrahydro-β-carbolines involves the ion of mass m/e 171 (base peak) which was formed in each instance by the loss of the alkyl radical adjacent to the nitrogen. The ions at m/e 144, 143 and 117 are formed by the loss of an HCN molecule from the base peak, followed by the subsequent loss of a hydrogen radical and a molecule of acetylene. Scheme 1 shows that the peak at m/e 117 could also arise by the successive loss of two HCN molecules from the base peak. An accurate mass determination of the m/e 117 peak in the mass spectrum of 1-methyl-1,2,3,4-tetrahydro-β-carboline (Ia) revealed that it was composed of two ions, C_8H_7N (67%) and C_9H_9 (33%).

The expulsion of an RNH radical from the molecular ion to give a diagnostic ion of mass m/e 156 for the six 1-alkyl-1,2,3,4-tetra hydro-β-carbolines has not been reported previously. The relative abundance of the m/e 156 ion varied (3% to 35%), however accurate mass measurements confirmed that in each spectrum a fragment ion of

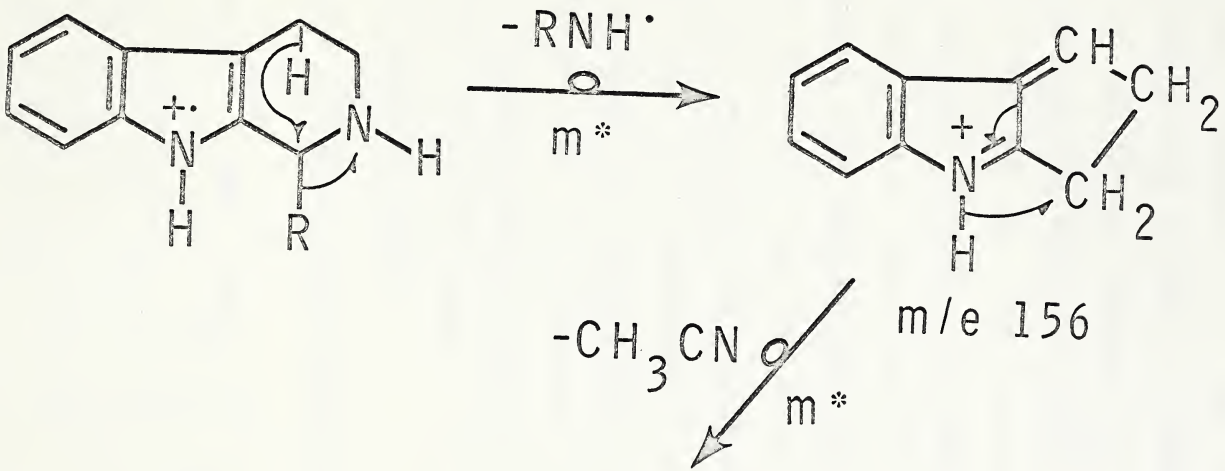


SCHEME 1

the composition $C_{11}H_{10}N$ was present. The formation of this ion would require the expulsion of a RNH radical from the molecular ion and this was supported by the observation of metastable ions in five of the six spectra. The presence of a metastable ion was not observed for 1-methyl-1,2,3,4-tetrahydro-β-carboline (Ia). A rearrangement, such as that shown in scheme 2, could account for the loss of the RNH radical and a subsequent loss of a $CH_3C{\equiv}N$ molecule to give a peak at m/e 115. It was shown that the deuterated 1-isobutyl-1,2,3,4-tetrahydro-β-carboline (V) had an abundant ion (11%) at m/e 157, whereas it was absent from the spectrum of If. This would account for the replacement of the hydrogen on the N-2 nitrogen by deuterium and then an expulsion of the C_4H_9DN radical.

In three instances the ion at m/e 156 was a doublet composed of $C_{10}H_8N_2$ and $C_{11}H_{10}N$ (Table 1). The origin of the $C_{10}H_8N_2$ ion is obscure, however it would appear to involve the loss of the alkyl side chain to form the 3,4-dihydro-β-carboline, followed by a ring rearrangement resulting in the expulsion of a methyl radical. This has been shown to occur with 1,2,3,4-tetrahydroquinoline (65).

The presence of a retro-Diels-Alder fragmentation in alkaloids has been well established. Ajmalicine (43), eburnamine (66), akuammicine (67) and anhydrousdihydrolycopodine (68) all fragment by a retro-Diels-Alder mechanism. The proposed mechanism of 1-methyl-1,2,3,4-tetra-



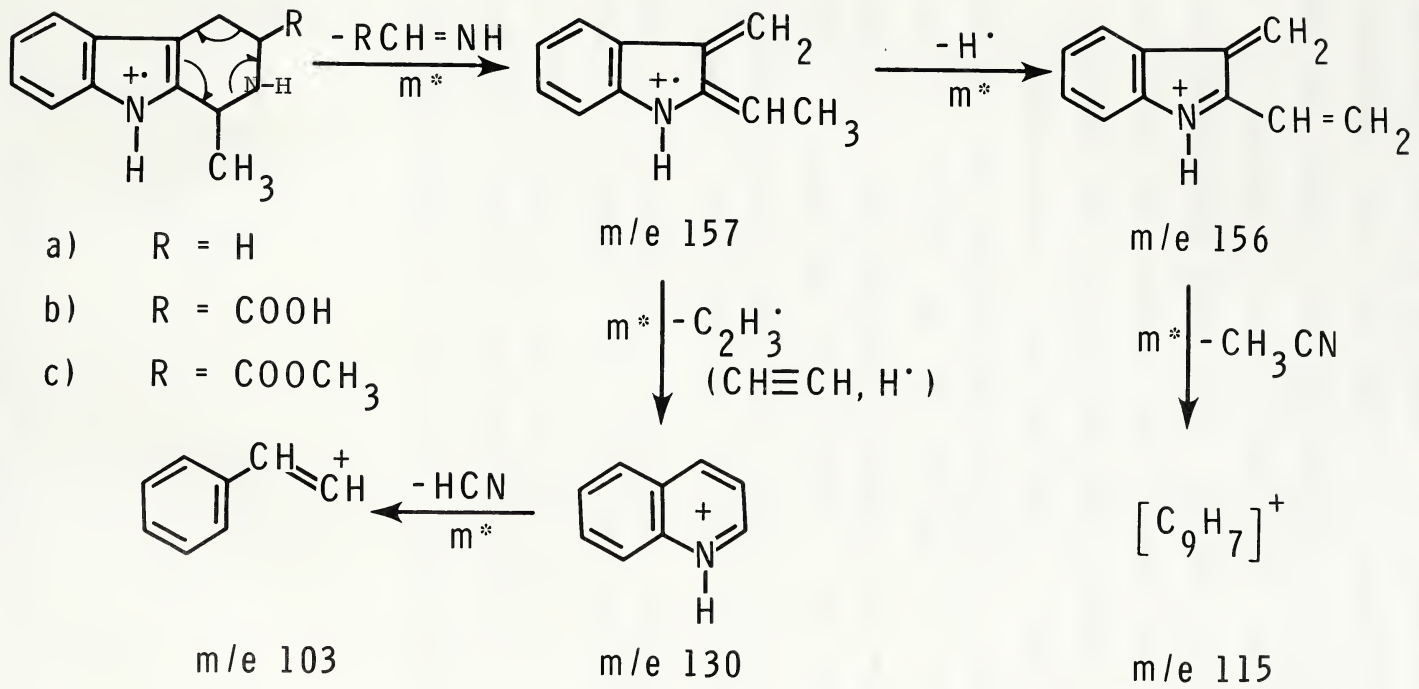
m/e 115

SCHEME 2

hydro- β -carboline (Ia), the acid (Ig) and the ester (Ih), involves a retro-Diels-Alder fragmentation to give the ion of mass m/e 157. This ion was the base peak in the spectrum of Ig and Ih. Scheme 3 shows that the ion at m/e 157 further fragments by a loss of a H radical followed by the loss of a $CH_3C{\equiv}N$ molecule to give ions at m/e 156 and 115. The ion at m/e 157 can fragment by the loss of a H radical, an acetylene molecule and a HCN molecule to give ions at m/e 156, 130 and 103. These fragments were substantiated by the presence of appropriate metastable ions and by an accurate mass measurement of the m/e 157 ion, confirmed as $C_{11}H_{11}N$. The ion at m/e 171 in the spectrum of 1-ethyl-1,2,3,4-tetrahydro- β carboline (Ib) could result from a retro-Diels-Alder mechanism since its molecular ion is m/e 200 and a loss of a $CH_2{=}NH$ molecule can give an ion at m/e 171 of $C_{12}H_{13}N$. However, an accurate mass of this ion revealed that it was entirely $C_{11}H_{11}N_2$.

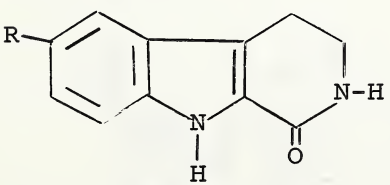
Table 1. Accurate Mass Determinations of m/e 156 Ion

Compound	%Rel. Abund.	Identity $C_{11}H_{10}N$	Identity $C_{10}H_8N_2$
Ia	35	100%	-
Ib	8	90%	10%
Ic	17	100%	-
Id	3	25%	75%
Ie	7	33%	66%
If	6	100%	-


-RCH=NH
m*
-H·
m*
m/e 157
m/e 156
I a) R = H
b) R = COOH
c) R = COOCH3
m* -C2H3·
(CH≡CH, H·)
m* -CH3CN
-HCN
m*
m/e 103
m/e 130
m/e 115
[C9H7]+

SCHEME 3

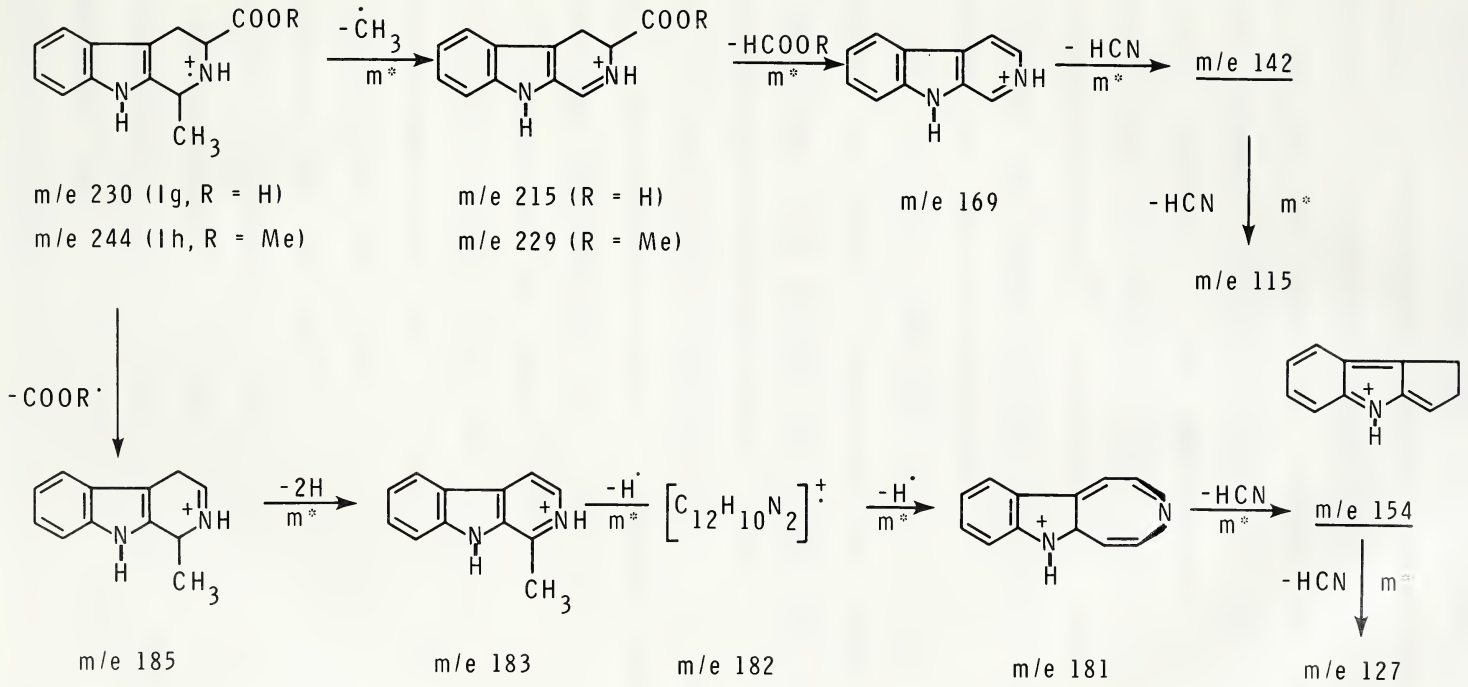
The acid (Ig) and the ester (Ih, Figure II) can fragment by pathways other than that of the retro-Diels-Alder. It is known that aromatic carbonyl compounds can undergo facile β -cleavage to give a loss of the hydroxyl and then the carbonyl group (69). However, this did not occur with Ig or Ih. Instead the molecular ion for the ester (m/e 244) and the acid (m/e 230) fragment by the loss of a COOR radical or by the loss of a methyl radical. The loss of a COOR radical gave an ion at m/e 185 followed by ions at m/e 183, 182, 181, 169 and 115 which were all supported by the presence of appropriate metastable ions and by accurate mass measurements. The loss of a methyl radical gave an ion at m/e 229 for Ih and 215 for Ig, followed by ions at m/e 169, 143 and 115, were also supported by the presence of appropriate metastable ions. The presence of abundant ions at m/e 185 and 183, in the spectra of the acid (Ig) and the ester (Ih) are diagnostic since such ions were not found in the mass spectra of the other tetrahydro- β -carbolines investigated (scheme 4).



II

	R
a)	H
b)	CH_3O

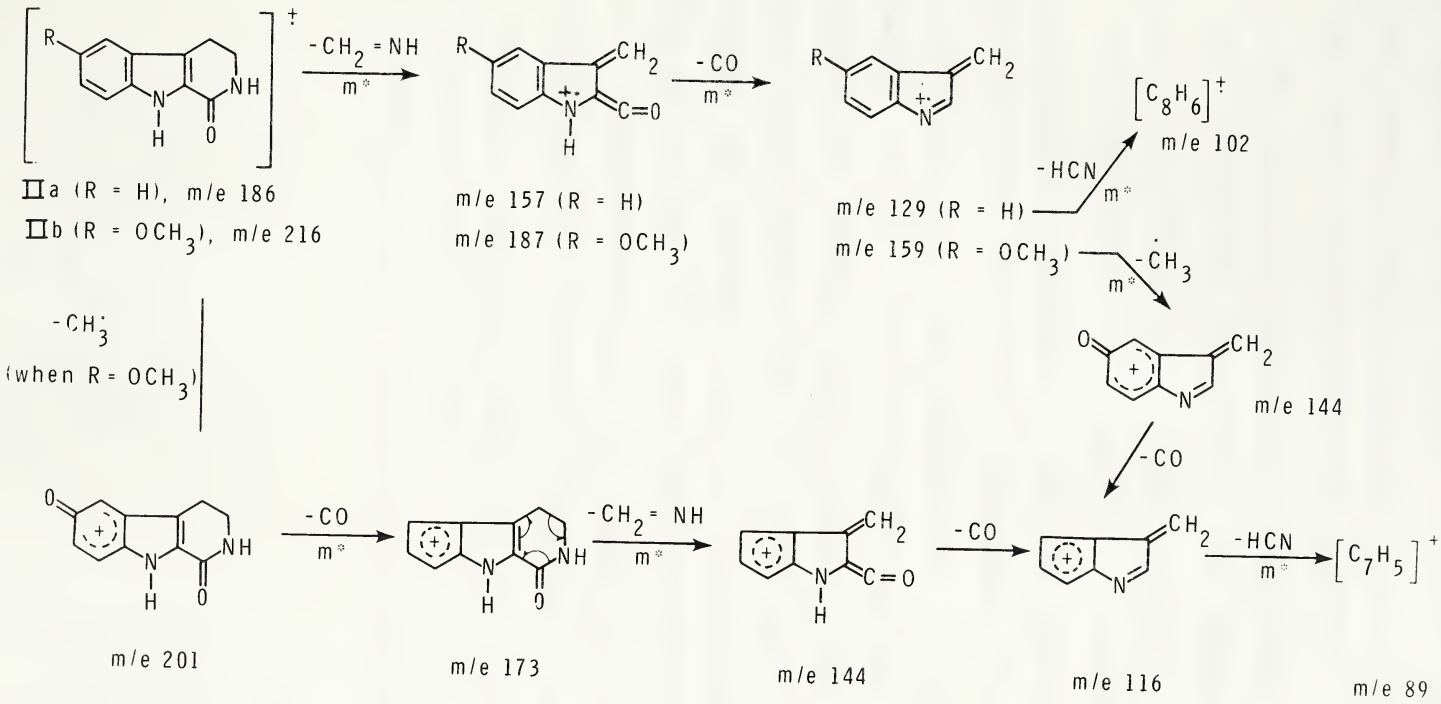
1,2,3,4-tetrahydro-1-oxo- β -carboline (1-tetrahydronorharmanones)


COOR
-·CH3
m*
COOR
-HCOOR
m*
- HCN
m*
m/e 142
m/e 230 (Ig, R = H)
m/e 244 (Ih, R = Me)
m/e 215 (R = H)
m/e 229 (R = Me)
m/e 169
-HCN
m*
m/e 115
-COOR·
-2H
m*
-H·
m*
[C12H10N2]+·
-H·
m*
-HCN
m*
m/e 154
-HCN
m*
m/e 185
m/e 183
m/e 182
m/e 181
m/e 127

SCHEME 4

Clungston and MacLean (70) showed that in N-methyl-2-quinolone and N-methyl-4-quinolone the carbonyl group was involved in the initial fragmentation of the molecule. Reisch, Pagnucco and Jantos (71) showed that quinolone alkaloids fragment initially through the loss of a carbonyl group. However, in the spectrum of 1,2,3,4-tetrahydro-1-oxo-β-carboline (IIa) and 1,2,3,4-tetrahydro-6-methoxy-1-oxo-β-carboline (IIb, Figure II) the first major fragment was formed through the loss of a CH_2=NH molecule followed by the loss of a CO molecule to give the base peak at m/e 129 for IIa and 159 for IIb (scheme 5). The formation of the $(M-29)^+$ ion was due to a retro-Diels-Alder mechanism. The ion m/e 187 in the spectrum of 1,2,3,4-tetrahydro-6-methoxy-1-oxo-β-carboline was confirmed, by accurate mass measurements of the ion, to be $C_{11}H_9NO_2$. This would be shown if a CH_2=NH molecule was expelled from the molecular ion. For 1,2,3,4-tetrahydro-1-oxo-β-carboline (IIa) the $(M-29)^+$ ion was decomposed further by the loss of CO and HCN molecules. 1,2,3,4-tetrahydro-6-methoxy-1-oxo-β-carbolines (IIb) also lost a CO molecule from the $(M-29)^+$ ion, however it then lost a CH_3 radical from the methoxy group followed by the expulsion of a CO molecule to give a peak at m/e 116.

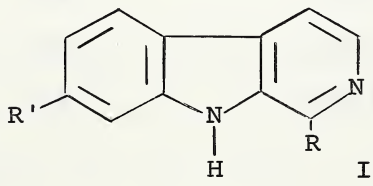
The presence of a methoxy group in 1,2,3,4-tetrahydro-6-methoxy-1-oxo-β-carboline (IIb) influenced the fragmentation modes of this compound. Scheme 5 depicits the mechanism proposed for the formation of ions appear-


-CH2 = NH
m*
-CO
m*
-HCN
m*
-CH3
m*
-CH3
(when R = OCH3)
-CO
m*
-CH2 = NH
m*
-CO
-HCN
m*
IIa (R = H), m/e 186
IIb (R = OCH3), m/e 216
m/e 157 (R = H)
m/e 187 (R = OCH3)
m/e 129 (R = H)
m/e 159 (R = OCH3)
[C8H6]+
m/e 102
m/e 144
m/e 201
m/e 173
m/e 144
m/e 116
m/e 89
[C7H5]+

SCHEME 5

ing at $(M-15)^+$, $(M-43)^+$, $(M-72)^+$, $(M-100)^+$, and $(M-127)^+$. Loss of a methyl radical would give rise to the peak at m/e 201. This was followed by the loss of CO, CH_2=NH, CO and HCN molecules respectively to give peaks at m/e 173, 144, 116 and 89. An accurate mass measurement of the m/e 173 ion showed that it was $C_{10}H_9N_2O$.

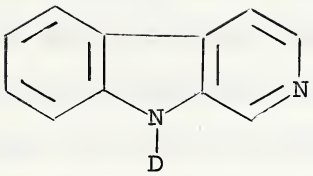
The mass spectra of simple β -carbolines have not been thoroughly investigated. The mass spectral fragmentations of the more complex tetrahydro- β -carbolines (43) have resulted in the formation of β -carbolines fragments. However, the mass spectral pathway of these fragments have not been further studied. The most extensive investigation of the β -carbolines was studied by Biemann (62) on three 1-alkyl- β -carbolines. The spectra of all four β -carbolines and the two deuterated β -carbolines (Figure III) showed intense molecular ion peaks, and the significant fragmentation was the loss of twenty-seven mass units (HCN) from the $(M-1)^+$ ion. These results are analogous to those which have previously been observed for quinoline, isoquinoline and pyridine (72).



III

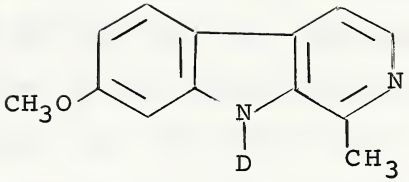
	R	R'
a)	H	H
b)	CH_3	H
c)	CH_3	OH
d)	CH_3	OCH_3

β - Carbolines



VI

Deuterated Norharmane



VII

Deuterated Harmine

Aromatic molecules and molecules containing conjugated systems commonly give rise to doubly charged ions because of the π-electron systems present (73). Doubly charged ions of significant strength are shown by the four β-carbolines and the two deuterated β-carbolines (Table 2).

Table 2. Doubly Charged Ion of β-Carbolines

Compound	Location (m/e)	Percentage
IIIa	84.0	3
VI	84.5	7.5
IIIb	91.0	17.6
IIIc	99.0	12.1
IIId	106.0	13.2
VII	106.5	5.2

The major fragmentation pathway of norharmane molecular ion (IIIa) was the successive loss of two HCN molecules to give abundant ions at m/e 141 and 114. The second fragmentation pathway was formed by the expulsion of a hydrogen atom from the molecular ion. The $(M-1)^+$ ion fragmented by losing two HCN molecules to give abundant ions at m/e 140 and 113. In the spectrum of deuterated norharmane (VI), a metastable ion was observed at m/e 167.0 corresponding to the loss of a hydrogen radical from the molecular ion. This indicates that the hydrogen atom expelled from norharmane was derived at least in part from locations in the molecule other than from the nitrogen atom.

The harmane (IIIb) molecular ion m/e 182, behaved similarly by losing successively a H radical and two HCN molecules to give abundant ions at m/e 181, 154 and 127. However, there is no indication that the molecular ion fragmented without first losing a H radical, such as occurred with norharmane.

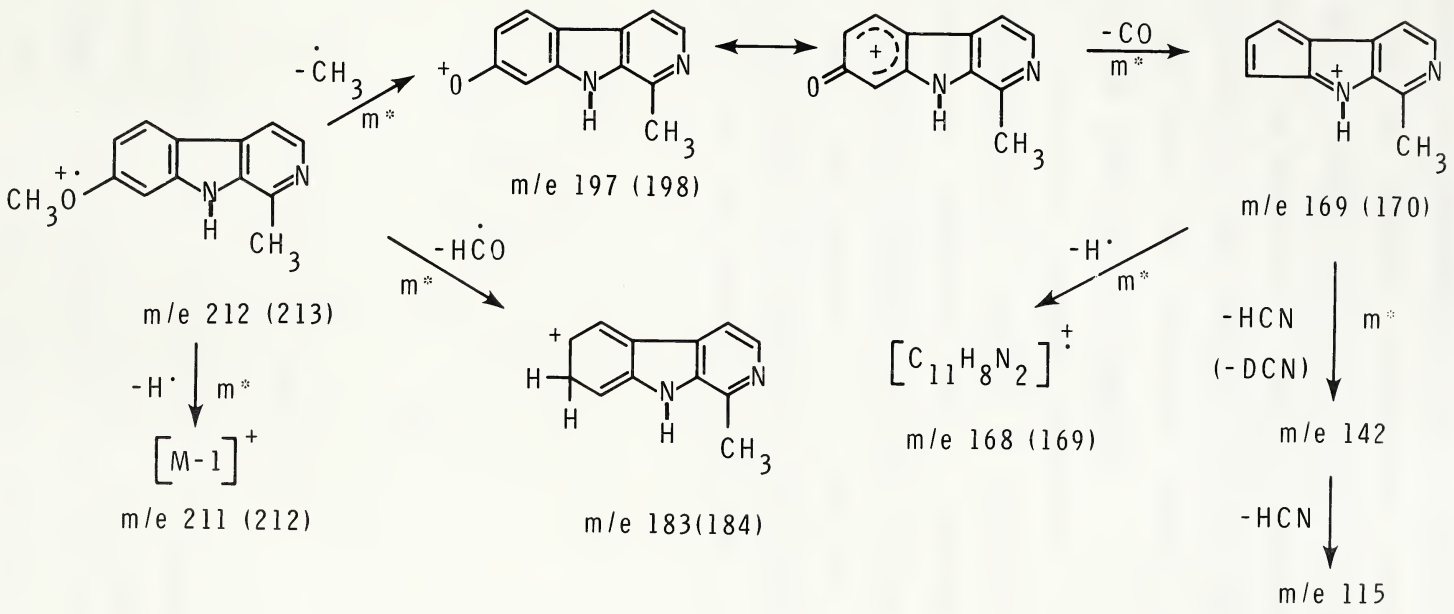
Harmol (IIIc) behaved similarly to norharmane and harmane. There was a loss of a H radical from the molecular ion followed by the loss of two HCN molecules and a CO molecule from the $(M-1)^+$ ion to give abundant ions at m/e 197, 170, 143 and 115. The peak at m/e 170 was confirmed by accurate mass to be $C_{11}H_8NO$. This would indicate that a CO molecule was not expelled from the molecular ion, but rather a H radical followed by a HCN

molecule. The ion of m/e 115 was shown by accurate mass measurement to be C_9H_7, formed from the m/e 170 ion by successive loss of CO and HCN molecules.

The major peaks in harmine (IIId) may be explained on the basis of the fragmentation mechanism outlined in scheme 6. This fragmentation mechanism is very similar to that of the oxygenated quinolines (70), although the major pathway occurs through the loss of a methyl radical from the methoxy group rather than through a loss of a H radical.

In scheme 6 a mechanism is proposed for the formation of ions appearing at m/e 197, 169, 142 and 115 in the mass spectrum of harmine. The loss of the methoxy methyl radical followed by expulsion of carbon monoxide is the usual mode of fragmentation of phenylmethyl ethers (74), and is also observed in the mass spectrum of harmine. This is followed by the subsequent loss of two HCN molecules.

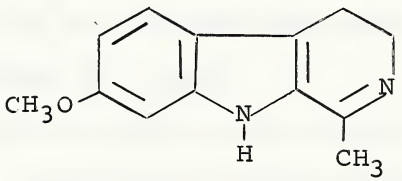
The spectrum of harmine also shows intense peaks for the molecular ion, as expected for aromatic compounds, and $(M-1)^+$ and $(M-29)^+$ ions. The methoxy group is involved in the formation of these fragment ions and this formation has also been reported in the mass spectra of deuterated 2 and 8-methoxyquinolines (74). The (M-1) peak is formed by the loss of one of the methyl hydrogens, and the (M-29) fragment is formed by the loss of a CHO radical.


-·CH3
m*
m/e 197 (198)
-CO
m*
m/e 169 (170)
m/e 212 (213)
-·HCO
m*
-H·
m*
[M-1]+
m/e 211 (212)
m/e 183(184)
-H·
m*
[C11H8N2]+·
m/e 168 (169)
-HCN
(-DCN)
m*
m/e 142
-HCN
m/e 115

SCHEME 6

Harmine was deuterated to give a molecular ion of m/e 213. The mass spectral fragmentation pathway was the same as for harmine except the fragments were one unit higher. The deuterated indole nitrogen was lost by the expulsion of a DCN molecule from m/e 169 to give m/e 142. The ion at m/e 142 is also found in the mass spectrum of the undeuterated compound.

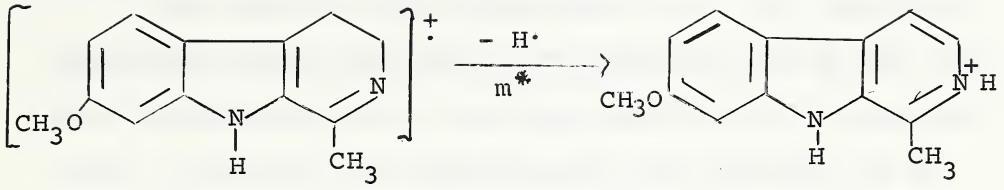
Very little work has been carried out on the mass spectral fragmentation of dihydro- β -carbolines or related compounds. Ajmalicine (43), a complex tetrahydro- β -carboline, fragments to form a dihydro- β -carboline with a peak at m/e 170. A loss of a hydrogen atom from the dihydro- β -carboline ring system to give a β -carbolinium ion occurred at m/e 169.



IV

3,4-dihydro- β -carboline

The molecular ion of harmaline (IV, Figure II) was the base peak and the $(M-1)^+$ ion was only slightly less abundant (98%). The conversion of the molecular ion m/e 214 to the $(M-1)^+$ ion can be depicted as shown in scheme 7.



m/e 214 m/e 213

Scheme 7

A loss of a CH_3 radical, a CO molecule and a HCN molecule occurred from both the m/e 214 and m/e 213 ions giving rise to the ions m/e 199, 171 and 144; and 198, 170 and 143 respectively. The $(M-1)^+$ ion also fragments by the loss of a HCN molecule followed by the expulsion of a CO molecule to give peaks at m/e 186 and 143. This was substantiated by the presence of an appropriate metastable ion and by an accurate mass measurement of the m/e 143 ion, confirmed as $C_{10}H_9N$.

Other minor fragmentations occurred in the spectrum of harmaline. From accurate mass determinations of peaks at m/e 171 and 172, it was shown that m/e 171 consisted of two ions, $C_{11}H_{11}N_2$ (75%) and $C_{11}H_9NO$ (25%). Similarly, the m/e 170 peak was a doublet of $C_{11}H_{10}N_2$ (75%) and $C_{11}H_8NO$ (25%).

EXPERIMENTAL

Mass spectra were determined by Dr. A.M. Hogg and associates using the direct probe method with an AEI MS-9 mass spectrometer at an ionizing potential of 70 electron volts. Accurate mass measurements were carried out by the peak matching method. All spectra are plotted in terms of relative abundance with the most intense peak in the spectrum being taken as 100%. Only those peaks with an intensity equal to or greater than 2% of the most intense peak are recorded.

Harmane, tetrahydroharmane-3-carboxylic acid, 6-methoxy-1-tetrahydronorharmanone, 1-tetrahydronorharmanone and harmol were obtained from the Aldrich Chemical Co., Milwaukee, Wisconsin. Norharmane, harmine and harmaline were purchased from the City Chemical Co., New York, N.Y., and tetrahydroharmane was purchased from K and K Laboratories Inc., Plainview, N.Y. The purity of these compounds was varified by taking the melting points.

Methyl ester of tetrahydroharmane carboxylic acid

Tetrahydroharmane carboxylic acid (Ih) was converted to the corresponding methyl ester by heating under reflux 100 mg of tetrahydroharmane carboxylic acid, 1 ml of H_2SO_4 and 10 mls of methanol for 13 hours. The reaction mixture was evaporated almost to dryness, treated with 10% sodium carbonate solution and extracted with ethyl ether. The ether solution was dried (Na_2SO_4),

evaporated and the residue crystallized from methanol-ether, mp 108-110°.

1-Ethyl-1,2,3,4-tetrahydro- β -carboline HCl (Ib)

An aqueous solution consisting of .270 g propionaldehyde (Eastman Organic Chemicals, Rochester, N.Y.), 10 mls of water and 1 ml alcohol was added to an ice cold aqueous solution of .200 g tryptamine hydrochloride (Eastman) to which hydrochloric acid was added to a pH 1. The mixture was stirred for half an hour at room temperature, heated to boiling for half an hour and kept at boiling for an hour. It was cooled, made alkaline with sodium carbonate, extracted with ethyl acetate, dried and evaporated on a flash evaporator. The residue was dissolved in methanol and a methanolic hydrogen chloride solution was added to give the salt, mp 237-238°. This procedure is a modification of the reported synthesis for 1 alkyl-1,2,3,4-tetrahydro- β -carbolines (30).

1-Propyl-1,2,3,4-tetrahydro- β -carboline HCl (Ic)

The procedure used was identical with that for Ib except .490 g n-butyraldehyde (Eastman) were added to .375 g tryptamine HCl, mp 270-272°.

1-Isopropyl-1,2,3,4-tetrahydro- β -carboline HCl (Id)

Four hundred and twenty-five mg of isobutyraldehyde (Matheson, Coleman & Bell, Norwood, Ohio) were added to .315 g tryptamine HCl, mp 258-260°.

1-Butyl-1,2,3,4-tetrahydro-β-carboline HCl (Ie)

Four hundred and ninety mg of n-valeraldehyde (Matheson, Coleman & Bell) were added to .375 g tryptamine HCl, mp 256-257°.

1-Isobutyl-1,2,3,4-tetrahydro-β-carboline HCl (If)

Seven hundred and twenty mg of isovaleraldehyde (Eastman) were added to .540 g of tryptamine HCl, mp 258-260°.

Deuterated Compounds

The deuterated compounds (1-isobutyl-1,2,3,4-tetrahydro-β-carboline HCl (V), norharmane (VI) and harmane (VII) were prepared by heating under reflux for 12 hours a solution of the appropriate β-carboline in dioxane containing deuterium oxide. The solution was cooled and evaporated to dryness on the flash evaporator. The N-H stretching bands between 3200 and 3500 cm^{-1} in the infrared spectra of the unlabeled β-carbolines were replaced by N-D stretching bands in the 2000-2400 cm^{-1} region in the deuterated compounds.

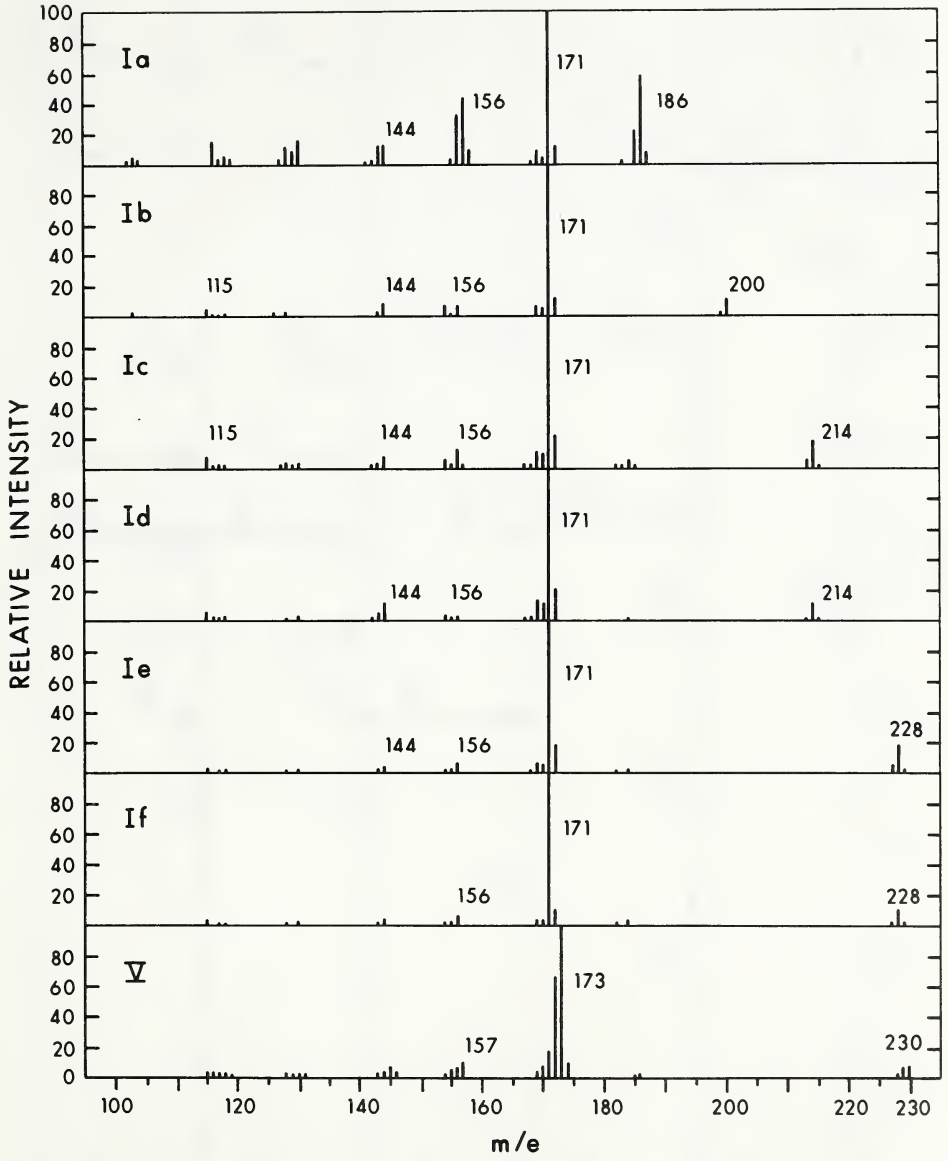


Figure I

Portions of the Mass Spectra of the 1-Alkyl-1,2,3,4-tetrahydro-β-carbolines (Ia-If) and Deuterated 1-Isobutyl-1,2,3,4-tetrahydro-β-carboline (V)

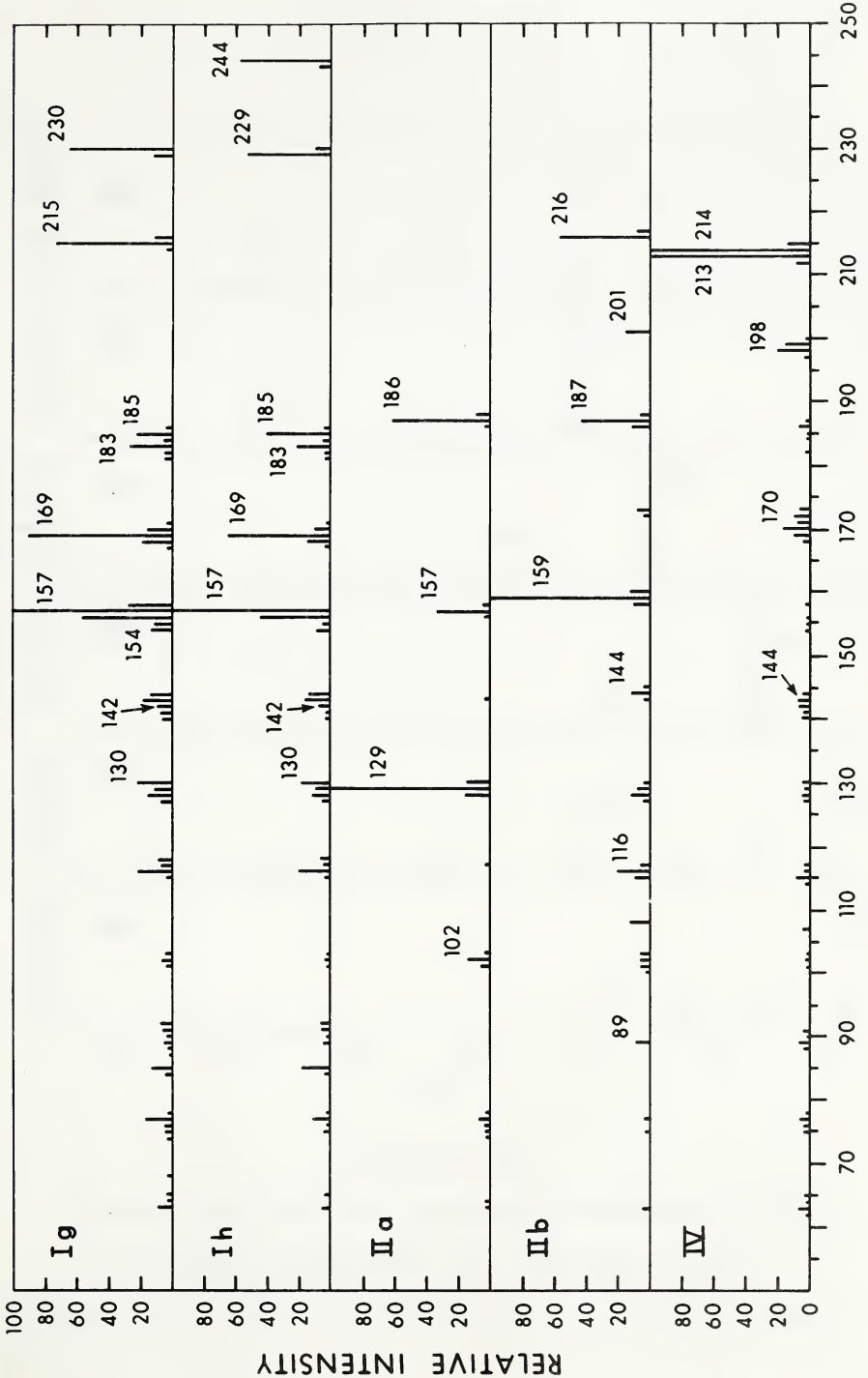


Figure II

Portions of the Mass Spectra of Tetrahydroharmane-3-carboxylic acid (Ig) and Methyl Ester (Ih), 1,2,3,4-Tetrahydro-1-oxo-β-carboline (IIa), 1,2,3,4-6-methoxy-1-oxo-β-carboline (IIb) and Harmaline (IV)

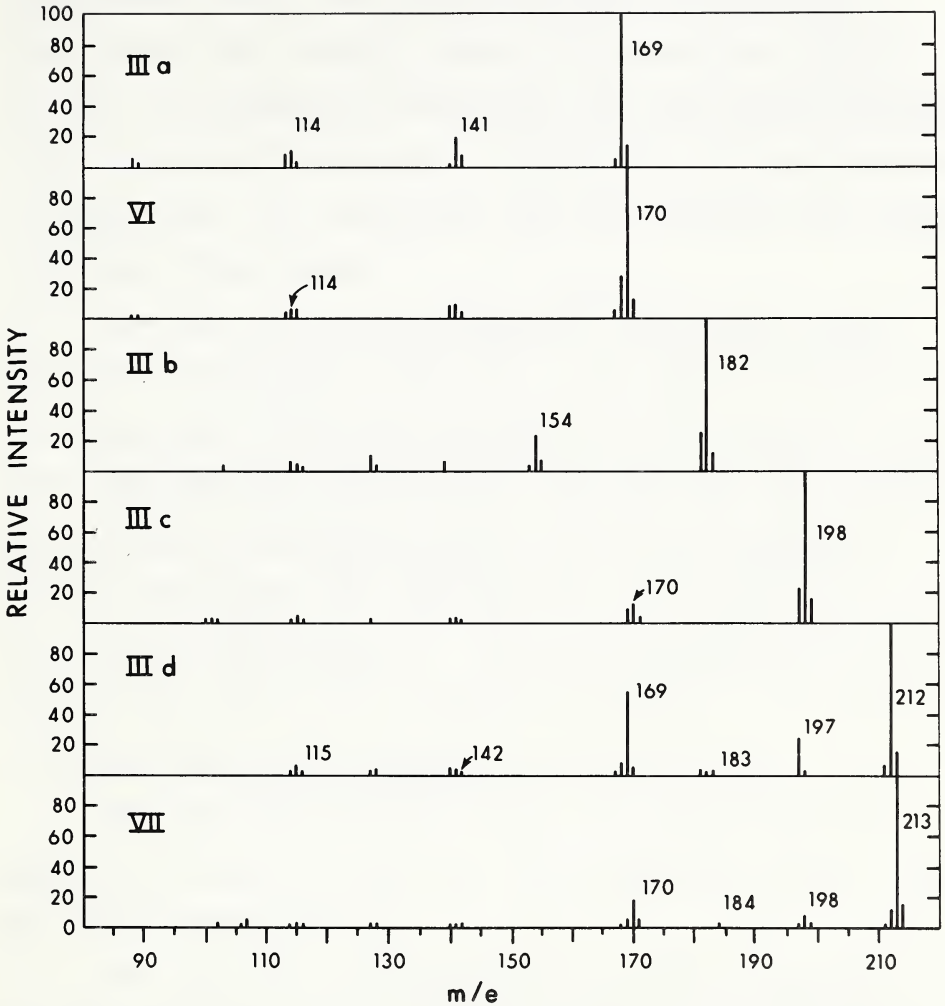


Figure III

Portions of the Mass Spectra of Norharmane (IIIa), Deuterated Norharmane (VI), Harmane (IIIb), Harmol (IIIc), Harmine (IIId) and Deuterated Harmine (VII)

SUMMARY

The mass spectra of eight 1,2,3,4-tetrahydro-β-carbolines (Ia-h), two 1,2,3,4-tetrahydro-1-oxo-β-carbolines (IIa and IIb), four β-carbolines (IIIa-d) and one 3,4-dihydro-β-carboline (IV) have been examined. Three deuterated compounds, 1-isobutyl-1,2,3,4-tetrahydro-β-carboline (V), norharmane (VI) and harmine (VII) have also been investigated.

The six 1,2,3,4-tetrahydro-β-carbolines (Ia-f) all show a base peak at m/e 171. This peak is formed by the expulsion of an alkyl radical from the molecular ion. This phenomena was confirmed by accurate mass measurements, the presence of metastable ions, and by deuterating a 1,2,3,4-tetrahydro-β-carboline (V) and comparing it to the undeuterated compound. The third fragmentation pathway applied only to the 1-methyl compounds Ia, Ig and Ih. A retro-Diels-Alder fragmentation was involved.

The acid (Ig) and the ester (Ih) fragmented by a retro-Diels-Alder, a loss of a COOH molecule, or by a loss of a CH_3 radical from C_1.

The two 1,2,3,4-tetrahydro-1-oxo-β-carbolines fragment by the loss of a CH_2=NH (M-29) molecule followed by the loss of a CO molecule to give the base peak at m/e 129 for IIa and 159 for IIb. The formation of the $(M-29)^+$ ion was due to a retro-Diels-Alder. The presence of a methoxy group in IIb also influenced the fragmentation pathway of this compound.

The β -carbolines and their deuterated analogues all indicate that the base peak and the molecular ion are the same. They also show doubly charged ions of significant strength at 1/2 m/e of the base peak. Norharmane (IIIa), deuterated norharmane (VI), harmane (IIIc) and harmol (IIIc) all fragment by the successive loss of a H radical followed by two HCN molecules. Norharmane also fragments by the loss of two HCN molecules from the molecular ion. Harmine fragments mainly by the loss of the methoxy methyl radical followed by an expulsion of carbon monoxide and the subsequent loss of two HCN molecules. It can also fragment by the loss of one of the methyl hydrogens to form the (M-1) peak and the (M-29) peak is formed by the loss of a CHO radical. The deuterated harmine has the same mass spectral fragmentation pathway as harmine only the fragments are one mass unit higher.

The spectrum of harmaline (IV) shows that the molecular ion and the $(M-1)^+$ ion are almost of the same intensity. The molecular ion loses a H radical to form the $(M-1)^+$ ion and they both fragment by a similar pattern through a loss of a CH_3 radical, a CO molecule and a HCN molecule. The $(M-1)^+$ ion also fragments by the loss of a HCN molecule followed by the expulsion of a CO molecule.

CONCLUSION

Although various aromatic and 1,2,3,4-tetrahydro-β-carbolines have been isolated from the Elaeagnaceae family, this constitutes the first report on the occurrence in this plant family of a 1,2,3,4-tetrahydro-β-carboline with an aliphatic side chain longer than one carbon atom. The isolation and characterization of 1-isobutyl-1,2,3,4-tetrahydro-β-carboline suggests that other indole or β-carboline alkaloids may be found which have their hypothetical biosynthesis from the condensation of tryptophan (tryptamine) intermediates and C_5 terpenoid unit.

This is the first report of an oxindole containing a tricyclic ring system. All of the other oxindoles isolated contain either a tetra or pentacyclic ring system. It is also the first report of an oxindole alkaloid in the Elaeagnaceae family. The presence of an oxindole and a 1,2,3,4-tetrahydro-β-carboline in the same plant may be biosynthetically important. In the laboratory, alkaloids which contain a 1,2,3,4-tetrahydro-β-carboline structure can be oxidatively converted into the corresponding oxindole. From a biosynthetic view point this could probably occur in the plant.

The synthesis and mass spectra of various 1,2,3,4-tetrahydro-β-carbolines was helpful in obtaining a more thorough knowledge of their fragmentation pathways. It was planned to synthesize the 1,2,3,4-tetrahydro-β-

carbolines with a C_4H_9 aliphatic side chain, however only n-butyl and isobutyl were prepared since the starting aldehydes for the t-butyl and 1-methyl propyl could not be obtained. The n-butyl and 1-isobutyl-1,2,3,4-tetrahydro-β-carbolines could not be distinguished by their mass spectra alone, and other spectrophotometric methods such as NMR were required. The mass spectra of various aromatic β-carbolines, 1,2,3,4-tetrahydro-1-oxo-β-carbolines and a 3,4-dihydro-β-carboline showed that the fragmentation pathways of each group of compounds were quite distinct from other groups. Although the structure of 1-isobutyl-1,2,3,4-tetrahydro-β-carboline could not be positively identified by its mass spectrum, the mass spectral evidence indicated that it was a 1,2,3,4-tetrahydro-β-carboline.

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